Proxies of Extra-Pair Behaviour

The influence of the Spatial, Temporal, and Social Setting on Patterns of Extra-Pair Paternity

DISSERTATION

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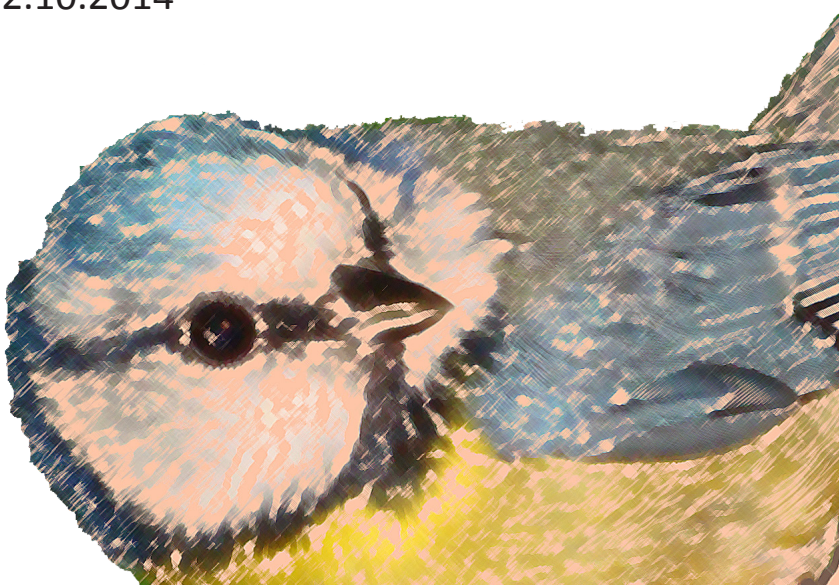

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List of Publications

Schlicht L., Girg A., Loës P., Valcu M., Kempenaers B. 2012. Male extrapair nestlings fledge first. *Animal Behaviour* 83(**6**):1335-1343.

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Valcu M. & Schlicht L. 2014. Expp: Spatial analysis of extra-pair paternity. R package version 1.1.

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Declaration of contribution as a first author for publications that are part of this thesis:

1. Schlicht L., Valcu M., Kempenaers B. 2014. Thiessen polygons as a model for animal territory estimation. *IBIS* 156(**1**): 215-219.

   LS initiated the project, performed the relevant analyses, and wrote the manuscript. LS and MV developed the project. LS, MV, and BK discussed the statistics, the scientific implications, and the manuscript.

2. Schlicht L., Valcu M., Loës P., Girg A., Kempenaers B. 2014. No relationship between female emergence time from the roosting place and extrapair paternity. *Behavioural Ecology* 25(3): 650-659.

   LS designed the experiment, organized and performed the relevant field work with the help of field assistants, performed all statistical analyses, and wrote the manuscript. LS, MV, and BK discussed the experimental setup, the scientific implications, the statistical methods, and the proposed manuscript. PL designed the electronic equipment, GA genotyped all blood samples, BK did the paternity analysis.

Declaration of contribution as a co-author for publications that are part of this thesis:

1. Valcu M. & Schlicht L. 2014. Expp: Spatial analysis of extra-pair paternity. R package version 1.1.

   LS and MV developed the basic ideas underlying this R-package (see chapter 2: Spatial patterns of extra-pair paternity: beyond paternity gains and losses). MV wrote the package. LS tested the package and wrote the help.

Declaration of contribution as a co-author for future publications that are part of this thesis:

1. Schlicht L., Valcu M., Kempenaers B. 2014. Spatial patterns of extra-pair paternity: beyond paternity gains and losses. *Journal of Animal Ecology, provisionally accepted (minor revisions)*.

   LS and MV mutually developed the idea and the behavioural and statistical framework. LS performed all analyses, and wrote the manuscript. LS, MV, and BK discussed in detail the statistical analyses, the validity of the method, the scientific implications, and the manuscript.

2. Schlicht L., Valcu M., Kempenaers B. 2014. Male extra-territorial behavior predicts extra-pair paternity in blue tits, *Cyanistes caeruleus*. *Behavioural Ecology, under review*.

   LS proposed and developed the project, performed the statistical analyses, and wrote the manuscript. LS, MV, and BK discussed the statistical methods, the scientific implications, and the proposed manuscript.

Ort, Datum	Lotte Schlicht	Prof. Kempenaers

ZUSAMMENFASSUNG

Viele Vogelarten sind monogam, aber es kommt zugleich häufig zu Paarungen außerhalb des Paarbundes (Griffith *et al.* 2002). In einer großen Zahl von Studien wurde bereits der adaptive Wert der aus diesem Verhalten hervorgehenden „Fremdvaterschaften", insbesondere für Weibchen, anhand einer Kosten-Nutzen-Analyse auf genetischer Ebene untersucht. Die Ergebnisse dieser Studien haben jedoch bisher nicht zu generalisierbaren Resultaten geführt (Akçay & Roughgarden 2007). Im Gegensatz dazu gibt es relativ wenig Informationen über verhaltensbegründete Kosten und Vorteile von Fremdvaterschaften, obwohl diese ein Verständnis der Mechanismen von Fremdverpaarungen erleichtern können. Die hier vorliegende Studie untersucht, wie zeitliche, räumliche, und soziale Faktoren beeinflussen wann, wo, und warum bestimmte Individuen Nachkommen mit einem anderen als ihrem sozialen Partner zeugen. Ich untersuche dies an der Blaumeise (*Cyanistes caeruleus*), einer Art, für die genetische Konsequenzen von Fremdvaterschaften bereits detailliert untersucht wurden, bei der die Kenntnisse im Bereich des außerpaarlichen Verhaltens selbst aber noch unvollständig sind.

In den ersten beiden Studien stelle ich einen neuartigen Ansatz zur Untersuchung von Fremdvaterschaften vor und validiere diesen. Fremdverpaarungen erfordern als Beteiligte sowohl ein Weibchen als auch ein Männchen und beide können beeinflussen, ob eine Paarung stattfindet. In dem von mir vorgeschlagenen Ansatz werden nicht nur Informationen über das Individuum, sondern auch über potentielle und realisierte außerpaarliche Partner mit eingebunden. Bei Arten wie der Blaumeise, deren Territorien nicht nur in einzelnen, sondern in allen Aspekten des Brutverhaltens (Nahrungssuche, Balz, etc.) genutzt werden, sind Interaktionen räumlich oft auf Individuen aus nahegelegenen Territorien beschränkt. Auch außerpaarliches Verhalten zeigt eine solche Beschränkung. Im vorliegenden Ansatz werden daher räumliche Verhaltensstrukturen explizit in das Modell mit einbezogen. Um solche räumliche Verhaltensinformationen für einen Langzeitdatensatz über Brutverhalten (die hier eingesetzen Daten von Blaumeisen umfassen 12 Jahre und 2 Populationen) zu erhalten, ist es notwendig, im Nachhinein die Position der Territorien der Brutpaare abzuschätzen. In der Ökologie werden zu diesem Zweck immer häufiger Thiessen-Polygone eingesetzt (z.B. Wilkin *et al.* 2006, Valcu & Kempenaers 2008). Im **1. Kapitel** (**Chapter 1)** belege ich daher zunächst die Gültigkeit einer solchen Abschätzung von Territorien mit Hilfe von Thiessen-Polygonen, basierend of 14 publizierten Studien, für die detaillierten Informationen über Territorien vorliegen. Im **2. Kapitel** (**Chapter 2**) stelle ich dann den neuartigen Ansatz im Detail vor. Anschließend wende ich diesen auf den genannten Langzeitdatensatz an, nutze dabei Thiessen-Polygone als Approximation für die räumliche Beschränkung von außerpaarlichem Verhalten und untersuche gleichzeitig die Korrelation relevanter Parameter mit außerpaarlichem Fortpflanzungserfolg. Meine Resultate bestätigen die Ergebnisse früherer Studien und zeigen, dass dieser Ansatz genutzt werden kann, um Hypothesen zu testen, die mit bisherigen Methoden nicht statistisch robust überprüft werden konnten.

Fremdverpaarungen können früh morgens stattfinden und aus einer Studie über die nahe

verwandte Kohlmeise (*Parus major*) geht hervor, dass Weibchen, die ihren Schlafplatz morgens früher verließen, mit größerer Wahrscheinlichkeit außerpaarliche Nachkommen hatten. Im **3. Kapitel** (**Chapter 3**) untersuche ich mit Hilfe von korrelativen Daten über vier Jahre diesen Zusammenhang für Blaumeisen. Darüber hinaus wurden die Aufstehzeiten von Weibchen über zwei Jahre hinweg experimentell manipuliert, um einen direkten kausalen Zusammenhang zwischen Aufstehzeiten und außerpaarlichem Fortpflanzungserfolg zu ergründen. Ich konnte keinen solchen Zusammenhang feststellen, jedoch führte das Experiment interessanterweise in den zwei Jahren zu entgegengesetzten Ergebnissen. Dies könnte ein Hinweis darauf sein, dass das Verhalten von Weibchen am frühen Morgen durchaus Relevanz für Fremdverpaarungen hat, dass jedoch eine Interaktion mit Umwelteinflüssen diese Zusammenhänge beeinflussen kann.

Im **4. Kapitel** (**Chapter 4**) beschreibe ich, dass Blaumeisen vor oder während der Legephase der Weibchen immer wieder die Nester fremder Paare besuchen. Für Männchen korrelierte dieses Verhalten auch mit ihrem außerpaarlichen Fortpflanzungserfolg. Es erwies sich nämlich, dass ein Männchen, welches das Nest eines Weibchens besucht, mit deutlich größerer Wahrscheinlichkeit außerpaarliche Nachkommen mit diesem Weibchen zeugt. Im Gegensatz zu einer früheren Studie an Blaumeisen (Kempenaers *et al.* 1992) zeigt dieses Ergebnis, dass nicht nur das Verhalten des Weibchens, sondern auch das des Männchens einen wichtigen Einfluss auf die außerpaarliche Fortpflanzung haben kann.

Im **5. Kapitel** (**Chapter 5**) betrachte ich abschließend das Balzverhalten der Blaumeisen. Dies erfolgt mittels Audioaufnahmen von Rufen, die speziell im Balzkontext eingesetzt werden (Bijnens & Dhondt 1984). Zunächst beschreibe ich das zeitliche Vorkommen dieser Rufe im Laufe der Brutsaison und im Tagesverlauf. Im nächsten Schritt vergleiche ich diese Daten mit früheren Studien, um zu verifizieren, dass Balzrufe tatsächlich als Maß für das Balzverhalten verwendet werden können. Anschließend untersuche ich dann speziell Balzrufe von Männchen, die in einem Kontext auftreten, der nahelegt, dass die Rufe nicht an das soziale Weibchen gerichtet sind. Es ist wahrscheinlich, dass diese (zu großen Teilen) außerpaarliche Balz anzeigen. Es gab keine Korrelation der „außerpaarlichen Balzversuche“ von Männchen mit ihrem außerpaarlichen Fortpflanzungserfolg, jedoch verloren Männchen mit vielen außerpaarlichen Balzversuchen weniger Vaterschaft in ihrem eigenen Nest. Dies könnte darauf hindeuten, dass die Qualität von Blaumeisenmännchen auf Verhaltensebene relevant für den außerpaarlichen Fortpflanzungserfolg ist.

In der allgemeinen Einleitung (**General Introduction**) beschreibe ich detailliert den biologischen Hintergrund dieser fünf Studien. Weiterhin diskutiere ich die Relevanz von Studien im Bereich der Forschung über Fremdvaterschaften, die anstelle der rein genetischen Betrachtung darüber hinaus auch das Verhalten untersuchen. In der allgemeinen Diskussion (**General Discussion**) erörtere ich die Implikationen dieser fünf Studien für außerpaarliches Verhalten: Das zeitliche Vorkommen von Balz und Paarungen im Tagesverlauf und über die Saison hinweg, das räumliche Vorkommen, die relative Wichtigkeit des Verhaltens von Männchen und Weibchen und welche Faktoren beeinflussen, dass es zu Fremdverpaarungen zwischen einem ganz bestimmten Männchen und Weibchen kommt.

Summary

Most of the bird species that have to date been studied perform copulations outside the pair-bond (Griffith *et al.* 2002). A large body of literature is concerned with the investigation of genetic costs and benefits of such extra-pair paternity, for females in particular. However, results remain inconclusive (Akçay & Roughgarden 2007). The costs and benefits of extra-pair paternity in the behavioural domain have received much less attention, although this knowledge may help to understand the mechanisms which drive the patterns of extra-pair parentage that we observe. Here, I aim to investigate how the temporal, spatial, and social setting influences when, where, and why individuals have extra-pair offspring with one another. I investigate these proxies of extra-pair paternity in the blue tit, *Cyanistes caeruleus*, a species for which studies on genetic costs and benefits of extra-pair paternity are abundant, but the knowledge on the behavioural level is still incomplete.

The first two studies propose and validate a novel approach for investigating extra-pair paternity patterns. Extra-pair paternity arises from extra-pair copulations. Therefore at least one male and one female are involved in the production of extra-pair offspring and they both can influence whether extra-pair copulations occur. The approach I propose therefore investigates not only an individual's extra-pair paternity levels, but it also includes information on potential and realized extra-pair mates. In species with all-purpose territories (such as the blue tit), interactions are often majorly limited to individuals with close-by territories. This reduces the spatial scale at which extra-pair paternity occurs. For this reason, the approach I propose also includes spatial information which allows explicit modelling of the spatial framework in which extra-pair paternity occurs. To implement this spatial framework it was necessary to post-hoc estimate territories in our long-term dataset on blue tit breeding behaviour spanning over twelve years of data and two independent study populations. To this end I used Thiessen polygons, a method of growing importance in the field of ecology (e.g. Wilkin *et al.* 2006, Valcu & Kempenaers 2008). In **Chapter 1**, I validate the use of Thiessen polygons as an approximation for territories by comparing mapped territories from 14 published studies with the respective computed polygons. In **Chapter 2**, I propose and describe the novel approach in detail, and apply it to the long-term dataset. I define the spatial framework using Thiessen polygons as an estimate for territory sizes and locations, and investigate the correlation of several parameters with extra-pair paternity simultaneously. In short, I find that the proposed approach validates previous findings and at the same time allows testing hypotheses which previously could not be robustly tested.

A study on great tits (*Parus major*) reported that females that emerged earlier in the morning from their roosting site were more likely to have extra-pair offspring. In **Chapter 3**, I test for this relationship using four years of correlational data. Additionally, I designed an experiment which advances female emergence times at dawn to robustly test for a direct causal link between emergence times and extra-pair paternity. I could not verify that emergence times immediately influenced female extra-pair paternity. The experiment led to opposite results in the two seasons, indicating that although female behaviour at dawn may have important consequences on extra-pair paternity, such effects

may interact un unexpected ways with environmental conditions.

In **Chapter 4**, I report on blue tits visiting other pair's breeding nestboxes shortly before or during the egg-laying period of the female. I describe this behaviour and for males also link the behaviour to extra-pair paternity. I find that males that visit the nestbox of a female other than their social mate largely increase their chances of having extra-pair offspring with that female. In contrast to a previous study (Kempenaers *et al.* 1992) this suggests that not only female but also male behaviour influences the patterns of extra-pair paternity that we observe.

Finally, in **Chapter 5** I study the courtship behaviour of blue tits using sound recordings of specific calls that are uttered during courtships and copulations (Bijnens & Dhondt 1984). I describe the occurrence of calls throughout the day and season and validate that these courtship rates are similar to previously reported values. I then focus on courtship calls of territorial males which are apparently not directed towards the social female. These are likely to be – at least partly – extra-pair courtships. I therefore correlate the number of such presumed "extra-pair courtships" to male extra-pair gains and losses. I find no effect on extra-pair gains, but interestingly, males that performed more "extra-pair courtships" lost less paternity. This could indicate that on the behavioural level male quality may be important for the patterns of extra-pair paternity that we observe.

In the **General Introduction** I present in detail the biological background for the five studies, and discuss the importance of studying extra-pair paternity not only from a genetic, but also from a behavioural perspective. In the **General Discussion**, I specifically address the behavioural implications of these five studies with regards to the daily timing, seasonal timing, and location of courtships or copulations. I then go on to discuss the importance of male vs. female behaviour in driving patterns of extra-pair paternity. Finally, I examine potential factors that may explain which individuals have extra-pair offspring with each other.

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General Introduction

> *"*[…] *Epigamic display* [= display after pair formation has taken place] *is in a different category* [than Darwinian sexual selection]*, since in monogamous territorial birds the female normally has no chance of seeing the epigamic display of any male except her mate, and the latter may have been selected some weeks or months earlier."*

This statement by Lack (1940) in a major review on pair formation in birds reflects the view at the time, that the "normal avian condition [is] monogamy" (Lack 1940). Yet already then reports of copulations outside the pair-bond (extra-pair copulations) existed (summarized in Lack 1940). Such observations were rare, however, and therefore considered unusual exceptions. Thus, it was thought that only one male is important for explaining events occurring at breeding. This male (Lack refers to it as the "mate") is the male temporally and spatially associated with the nest, engaging in pair bonding behaviour with the female that lays the clutch, and often providing care to the young in the nest; it is the social male.

Today we know that in many supposedly monogamous bird species, other males than the social mate (extra-pair males) quite regularly sire young in a nest. These extra-pair offspring occur in approximately 90% of the studied bird species (Griffith *et al.* 2002). Hence, in contrast to Lack's statement above, many females do have the opportunity to see displays of males other than their social mate even after pair formation has already taken place. Potentially, they may select additional sires for their offspring and even invest time and energy in order to do this (e.g. Buitron 1983, Cockburn *et al.* 2009). Thus, mating systems in birds are far more diverse than previously thought and explanations for events associated with breeding need to be much more complex. Indeed, behavioural and morphological traits may strongly be influenced by sexual selection via extra-pair matings. For this reason, extra-pair behaviour became a major field of research in behavioural ecology with the advancement of molecular tools 20 years ago.

By now, a large body of literature investigating different aspects of extra-pair behaviour exists. The majority of studies focus on the genetic costs and benefits of extra-pair matings for females. Results of these studies are sometimes contradictory and remain difficult to interpret (for reviews see Griffith et al. 2002, Akçay & Roughgarden 2007, Forstmeier *et al.* 2014). To some extent, the inconsistencies may be due to the lack of knowledge of the behaviour which leads to or is involved in extra-pair courtship and copulation.

Why study behaviour?

The study of extra-pair paternity started off as a field majorly relying on behavioural observations. Stressing this point, Hanski (1992) stated that in the study of extra-pair paternity there was "a general problem: the success of extra-pair copulations cannot be measured from direct observations, genetic techniques such as DNA fingerprinting are needed." Nowadays this view has been turned around: only a few years after this statement, molecular tools became readily available, and allowed the scientists to gather large amounts of data providing information on the existence, number, and distribution of extra-pair offspring. Due to the ease with which data on extra-pair offspring can be gathered nowadays, most studies no longer invest in laborious observations to also obtain data on the behaviour leading to the production of extra-pair offspring in first place.

Selection acts on variation in fitness, which solely depends on the actually existing offspring. Behavioural data are not needed to assess this. Hanski's (1992) "general problem" appears to be solved, because it is the success of extra-pair copulations that counts; for this information parentage analyses should be fully sufficient. However, variation in the number of offspring only leads to the evolution over generations if it is linked to the genetic makeup of individuals. At the same time, the context required for the final outcome of extra-pair parentage is not only a question of genes (Fig. 1). Important steps need to take place that depend not only on the genetic, but also on the social and environmental setting of a specific individual. For example, extra-pair young only occur if two opposite-sex individuals meet and copulate and only if this copulation leads to a fertilization. If paternity is assessed by sampling eggs or offspring, as is the case in most studies, fertilized ova also need successful embryonic development.

Identifying these components is important. For example, there is no point in relating the expression of a certain trait (e.g. an ornament) to fitness in terms of the number of extra-pair offspring, if some

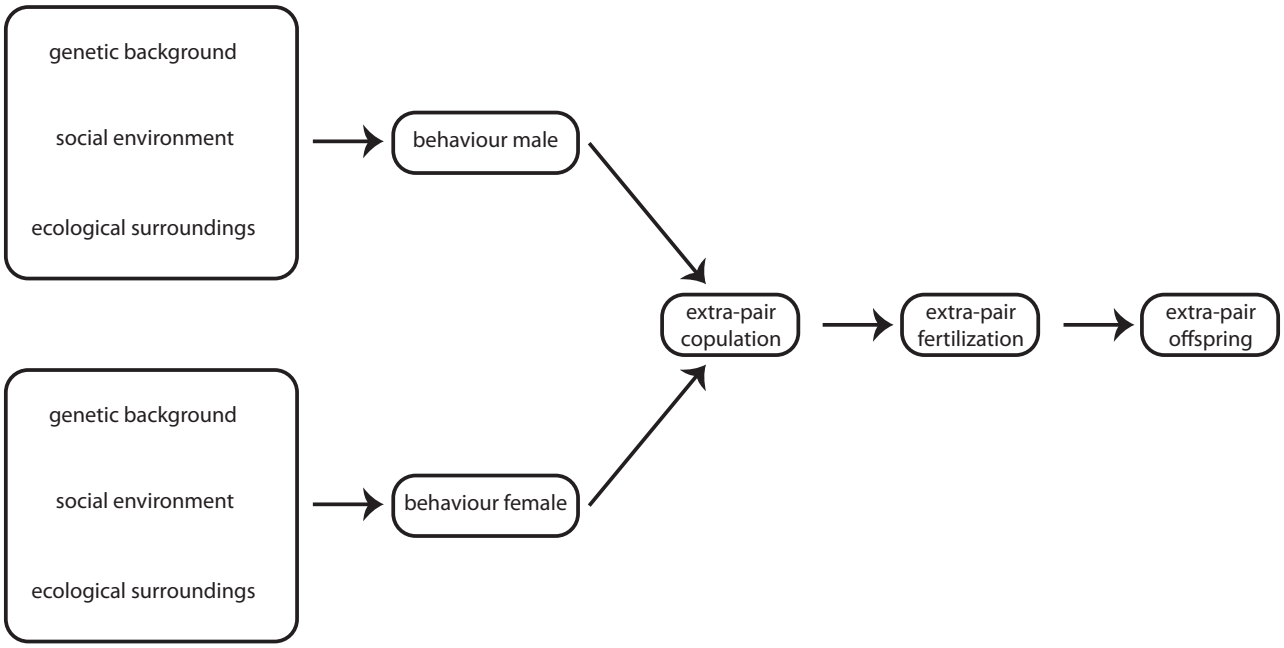


Fig. 1. Mechanisms which influence extra-pair paternity pattern. Most studies focus on the genetic mechanisms driving extra-pair paternity patterns measured by determining the paternity of offspring.

individuals lack the opportunity for extra-pair matings for reasons unrelated to their genetic makeup. Behavioural studies are the key to understand such mechanisms that can influence the observed patterns of extra-pair paternity and gain a broader understanding of associated costs and benefits. If extra-pair copulations are costly in terms of time and search costs, as is generally assumed (e.g. Dunn & Whittingham 2007), this leads to the expectation that, in evolutionary terms, females must benefit from extra-pair copulations, because else one would not expect this behaviour to exist (see Griffith *et al.* 2002 and references therein, but see Forstmeier *et al.* 2011). Behavioural studies can investigate where and when extra-pair courtships and copulations take place, which individuals are involved, and in what behavioural context. Occurrence in the context of, for example, behaviours serving self-maintenance would strengthen the hypothesis that extra-pair paternity is majorly a by-product of such behaviours. On the other hand, observation of forays directed at extra-pair mates would suggest a systematic behaviour beneficial to increase extra-pair mating opportunities. Information on these aspects of extra-pair paternity may help to better understand the outcome observed on the level of offspring parentage.

Which factors influence and determine the behaviour of individuals?

One major factor influencing an individual's behaviour is its genes (Fig. 1). Males gain additional offspring through extra-pair copulations, and this should lead to positive selection pressures for a genetic makeup that enhance extra-pair mating success in males. Female birds, on the other hand, are usually limited in their reproductive success by the number of eggs they can produce, and the benefits that select for extra-pair behaviour in females are therefore less clear. Indeed, a vast body of literature explores why, from an evolutionary perspective, female birds engage in extra-pair paternity (for references see Griffith *et al.* 2002, Akçay & Roughgarden 2007, Forstmeier *et al.* 2014). As mentioned above, active extra-pair behaviour may incur some costs, especially with regards to time and energy expenditure, and possibly also with regards to reduction of paternal care (Matysiokovà & Remeš 2013, for references see also Griffith *et al.* 2002). Predominant hypotheses why females are selected to engage in extra-pair behaviour are (for details and references see Griffith *et al.*, 2002 and Forstmeier *et al.* 2014):

- Fertility insurance hypothesis: copulation with multiple males may prevent production of infertile eggs in cases where the social male is sterile or his sperm cannot fertilize the female's ova for other reasons (Hasson & Stone 2009).

- Genetic diversity hypothesis: combining offspring of multiple paternity leads to greater genetic diversity in a nest, which may have benefits such as a better resistance against the sweep of a parasite (Yasui 1998).

- Genetic compatibility hypothesis: a partner genetically more compatible than the social mate may reduce hatching failure, or, in combination with the female genome, produce genetically fitter offspring (Puurtinen *et al.* 2009).

- "Good genes" hypothesis: A partner genetically superior to the social mate in terms of traits related to viability or mating success may produce genetically fitter offspring (Neff & Pitcher 2005).

- Direct benefits hypothesis: A female may gain immediate, material, non-genetic benefits from an extra-pair mating, such as nesting material or access to a foraging site (Price 1993).

- Indirect selection hypothesis: If female extra-pair behaviour has a genetic basis that is important also for the expression of male extra-pair behaviour, occurrence of extra-pair behaviour may be selected for in both males and females, if its benefits for males outweigh any immediate costs to females (Forstmeier *et al.* 2011).

In addition, extra-pair copulation may be selected against in females, but still manifest itself due to male coercion.

- Male coercion hypothesis: An extra-pair male may overpower the female and enforce copulation (mostly in waterfowl, Westneat & Stewart 2003).

Many studies have attempted to address these hypotheses, but findings are difficult to replicate, because they can vary between species, populations, study sites, and between study years (Akcay & Roughgarden 2007, Schmoll 2011, Kleven *et al.* 2011). In additionally, it is unlikely that there is one general explanation for the occurrence of extra-pair behaviour in females (Eliassen & Kokko 2008).

A second factor which influences an individual's immediate behaviour is its ecological surroundings (Fig.1). Globally, climate (predictable changes in e.g. temperature, precipitation), latitude, altitude, and connectivity (patchiness) of species' range contribute to temporal and spatial differentiation in conditions and resources. Locally, the biotic (plant and animal community) and abiotic habitat (e.g. weather) also introduce temporal and spatial structure, for instance in food availability, predator abundance, and the energy expenditure required. Such variation may affect behaviour of individuals, especially in terms of movement patterns (and thus encounter rates), and the time available for non-maintenance behaviours. Extra-pair behaviour can occur only during this time and only during encounter of individuals and is therefore expected to co-vary with these ecological parameters. The need to incorporate major environmental effects into the study of extra-pair paternity has long been postulated (Westneat & Stewart 2003), and by now evidence for such effects is growing (e.g. Johnsen & Lifjeld 2003, Spottiswoode & Møller 2004, Schmoll 2011, Bonier *at al.* 2014).

A third factor which clearly influences an individual's behaviour is its social environment. The social environment encompasses all potential (intraspecific) interaction partners, as well as their characteristics and behaviour. For instance, breeding density and breeding synchrony have often been proposed to influence extra-pair paternity via the availability of mating partners (e.g. Canal *et al.* 2012, Taff *et al.* 2013, see also Griffith *et al.* 2002 and references therein). Clearly, in the absence of potential extra-pair mates, no extra-pair copulations can take place. Not only the general availability, but also the behaviour and the characteristics of potential extra-pair mates may inhibit or favour the occurrence of extra-pair behaviour. If females indeed display mate choice for specific

extra-pair males, then their likeliness to have extra-pair offspring may largely depend on the quality of the available extra-pair mates (potentially in comparison with her social mate, Dias *et al.* 2009). Finally, the behaviour and the characteristics of the social partner may largely influence extra-pair mating opportunities. Males, for instance, often perform mate-guarding (e.g. Birkhead *et al.* 1992, Kempenaers *et al.* 1995, Johnsen *et al.* 2003) and interrupt extra-pair copulations of their social female if possible (Tarof & Ratcliffe 2000), thereby potentially reducing their mate's extra-pair mating opportunities. On the other hand, males may face a trade-off between mate-guarding and extra-pair behaviour, and may thus have little opportunities for extra-pair behaviour during their social female's fertile period (Westneat 1993, Johnsen *et al. 2003*, but Hasselquist *et al.* 1995, Kempenaers *et al.* 1995).

Given that most territorial birds have a limited home range (which often does not exceed a certain distance beyond the own territory, e.g. Naef-Daenzer 1994, Campioni *et al.* 2013), the spatial context in which interactions such as extra-pair courtships or copulations occur is often limited to an area that does not encompass the whole population. In consequence, population-wide analyses often render limited results. For many questions it is thus important to include information about the identity of potential interaction partners or the local density an individual experiences.

On the behavioural side, the three key factors listed above will be important determinants of extra-pair parentage. However, the sequence of behaviours leading up to extra-pair offspring ends at copulation. After this, post-copulatory processes (sperm competition, cryptic female choice, ejaculate-female-tract-interactions) become decisive. They provide the link between the observable behaviour of extra-pair copulations and the resultant pattern of extra-pair fertilizations. Finally, after fertilization, further aspects of the female physiology will modulate the viability of embryos and lead to the pattern of extra-pair offspring that is revealed by parentage analyses. Post-copulatory and post-fertilization processes will not be covered by this study. If a sufficiently large proportion of young is sampled, then the verified extra-pair paternity should correlate well with the extra-pair fertilizations that took place. Although relatively little is known about the potential for post-copulatory mate-choice, it appears likely that the rate of extra-pair fertilizations at least to some degree reflects the rate of extra-pair copulations (Birkhead & Møller 1992 pp. 152-154).

The factors discussed above (Fig. 1) and interactions among them determine an individual's propensity to have extra-pair offspring and influence whether, when, and where this happens. In the simplest case, however, for extra-pair young to originate, two individuals that do not form a social pair and are ready to mate have to meet, interact, and copulate. There are several behavioural questions that can be addressed in order to investigate why specific individuals may have extra-pair offspring with one another.

Seasonal timing. A successful extra-pair copulation can only occur if the female is fertile. If there is a trade-off between male mate-guarding and investing in extra-pair behaviour, and if mate-guarding is more important than obtaining extra-pair offspring, the male will only invest in extra-pair behaviour when his social female is outside of the window of her peak fertility (Birkhead & Møller 1992 p. 121,

but see Kempenaers 1997).

Daily timing. Extra-pair copulations may occur at specific times of the day, when one of the sexes is in a predictable location. For instance, at dawn the male advertises his location by singing while the female is often roosting in the selected nesting location. Extra-pair copulations may also occur preferentially immediately after egg-laying took place, when the chance of fertilization may be greatest (insemination window hypothesis, Birkhead & Moeller 1992 p.97).

Location. Males may foray into female territories and mate there, but females may also foray into male territories, with the copulations taking place there (for a review see Westneat & Stewart 2003). Such observations could suggest that sometimes the extra-pair partners come together due to active visits. In some species, the extra-pair mates may meet in a location outside both of their territories (e.g. Gray 1996), e.g. on common foraging grounds. In such a case, extra-pair mating could well be a by-product of foraging decisions. As discussed below, these questions may have major implications regarding the evolutionary mechanisms maintaining extra-pair behaviour.

Is the behaviour of one sex more important than that of the other? One sex could be relatively passively accepting extra-pair copulations, whereas the other sex invests actively into extra-pair behaviour. Such a pattern would suggest that it is selection on one sex that is driving evolution of extra-pair behaviour. Again, this would have major consequences for the debate of costs and benefits of extra-pair behaviour for males and females.

Who mates with whom? To date, most studies focus on the characteristics of an individual that could influence whether this individual has extra-pair offspring. However, as discussed in the previous paragraphs, an individual's propensity to invest into extra-pair behaviour may largely depend on the characteristics of available mates (their behaviour, their genetic "quality", etc.). For a better understanding why individuals engage in extra-pair behaviour it is thus important not only to know which males and which females have extra-pair offspring, but also which male and female have extra-pair offspring with one another.

The study species

This study focuses on the blue tit (*Cyanistes caeruleus*), one of the model species in avian ecology. Apart from the general knowledge about blue tit breeding behaviour (Box 1), many details of the biology of blue tits and particularly their breeding ecology are well studied, including genetic benefits of extra-pair paternity (e.g. Charmantier *et al.* 2004, Magrath *et al.* 2009, Parker 2013). Datasets are available to address questions which require a large amount of information, and experiments can robustly be designed to fit the biology of the species. Because blue tits readily accept nestboxes for breeding, they can be studied in the wild in a semi-standardized framework. The high density at which blue tits breed in nestbox populations provides sufficient sample sizes for experiments even within one or a few breeding seasons.

Box 1. The breeding biology of blue tits.

Blue tits are small (10-12 g), short-lived, non-migratory passerines which breed throughout Europe. They are most abundant in deciduous forests, especially oak-forests, but are also frequent garden-visitors. In summer, blue tits feed mostly on insects and spiders, in winter on seeds and fruits. As many other cavity-nesting species, they readily accept nestboxes provided by humans for breeding. During the breeding season, they generally form monogamous pairs, although some males may be polygynous (3% - 20% of males, Dhondt 1987, Kempenaers 1994b, Schlicht & Kempenaers 2013). Their breeding season (April through May) is relatively short; after nest-building (1), they usually produce one clutch of on average ten eggs (2-3), which is then incubated (4) for about two weeks. Once the first offspring hatch (5), the nestlings stay in the nest for around three weeks (6-7) under the care of both parents. After fledging (8-9) they remain with the family assumedly for another two weeks. Blue tits generally do not produce second clutches after the first brood has fledged; replacement clutches due to nest failure regularly occur. Therefore, the period from nest-building to independence of the offspring usually last around 10 weeks, usually spanning across April and May (compiled from Perrins 1979 and Cramp & Perrins 1993 pp. 224-248).

Blue tits form stable pair bonds throughout one breeding season, during which they usually raise one brood together (Perrins 1979 pp. 160-163, p. 183). They have an intermediate rate of extra-pair paternity; about half of all broods contain at least one extra-pair offspring (usually 1 – 2 in a clutch of 10) and around 10% of all young are extra-pair (e.g. Kempenaers 1997, Delhey *et al.* 2003). This regular occurrence of extra-pair paternity makes it feasible to study extra-pair behaviour in blue tits both from an observational (frequent enough to observe) and from a statistical point of view (balanced design).

Basic knowledge on extra-pair behaviour is available from one blue tit population (Kempenaers *et al.* 1992, Kempenaers 1994a, Kempenaers *et al.* 1995, Kempenaers 1997, Kempenaers *et al.* 1997). In this study, I use this knowledge to design specific experiments and data collection protocols tailored to the study system.

The scope of this study

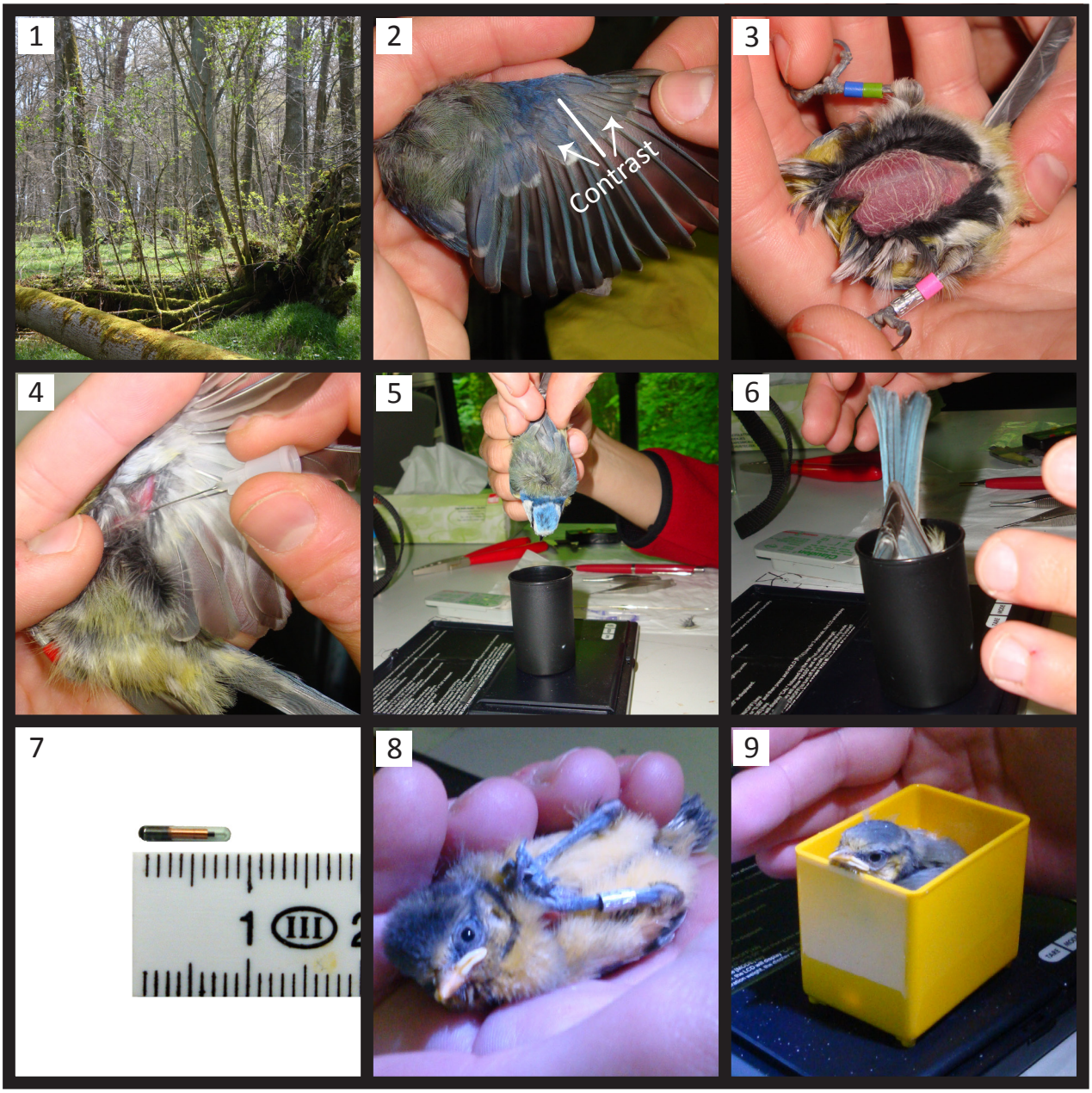
This study aims to address the mechanisms that explain temporal and spatial patterns in events of extra-pair parentage and their relationship to each other. Understanding how some individuals (but not others) come to mate with each other outside of the pair-bond requires a basic understanding of the circumstances under which such an event happens. To this end, all but one of the studies presented in this work address at least one of the questions stated below. Chapter 1 does not directly address any of these questions, but defines and validates the spatial framework in which extra-pair behaviour occurs, and thereby provides a basis for Chapter 2. Information regarding the basic methods of this study is presented in Box 2.

Seasonal timing. From the female point of view, extra-pair fertilizations can only occur while the female is fertile. In birds, the female fertile period generally starts a several days before egg laying commences and ends ca. 24 hours before the last egg of the clutch is laid (Birkhead & Møller 1992 p. 63, p. 66-67). In contrast to other species, blue tits do not stop copulating with the social partner once egg laying starts, but instead continue throughout the period of egg laying (Vedder *et al* .2010). Regarding extra-pair copulations, two previous studies reported that blue tit females restrict this behaviour to the days before or during early egg-laying (Magrath *et al.* 2009, Vedder *et al.* 2010), potentially because egg laying is time-consuming. Additionally, the presence of eggs in the nest may stimulate female incubation behaviour and thereby reduce extra-pair behaviour (Vedder *et al.* 2010). The male should time his extra-pair courtships such that the courted female is fertile. At the same time, male blue tits often invest heavily in mate-guarding, and may thus face a trade-off between guarding the social female (especially during peak fertility) and investing in extra-pair courtships (Dias *et al.* 2009, but Kempenaers 1997).

To understand how these constraints shape patterns of extra-pair paternity, I will therefore address the following questions. (1a) Do males adjust their investment into extra-pair behaviour in accordance with their social mate's fertility status (Chapter 4)? (1b) Do females adjust their investment into extra-

Box 2. Methods used in this study.

This study is mainly based on one population of blue tits in an unmanaged mixed-deciduous oak (1) forest close to Landsberg am Lech, Germany. The population was established in 2007 by supplying 277 small-holed nestboxes at two meters height. These nestboxes cannot be used for breeding by the larger tit species (e.g. great tits). Since 2007, the breeding behavior is monitored for all breeding pairs, including nest-building, the date of first egg, clutch size, hatching date, and fledging date. Additionally, all parents are caught when feeding at the nest, aged (2; based on plumage characteristics), sexed (3; brood-patch present/absent), banded (3; one metal band, three color bands), blood sampled (4; for paternity analysis), measured (5-6 body mass, tarsus length, wing length), and equipped with a small electronic transponder tag (7), which is automatically registered whenever an individual approaches a nestbox inside the study area. The nestlings (8) are banded (metal band only), blood sampled (for paternity analysis), and measured (9 body mass, tarsus length) when the oldest nestling of a brood is 14 days old.



pair behaviour in accordance with their own fertile period (Chapter 4)? (2) Do males adjust their investment into extra-pair behaviour in accordance with the potential extra-pair female's fertility status (Chapter 4)? (3) Do extra-pair couples form between males and females that are relatively asynchronous in their social breeding attempts (Chapter 2)?

Daily timing. The occurrence of extra-pair behaviour may be distributed evenly throughout the day. This should be the case in particular, if extra-pair matings result from random encounters of individuals only (Brommer *et al.* 2007). Some previous studies suggest, however, that extra-pair behaviour peaks in the early morning hours (Kempenaers 1994a, Double & Cockburn 2000). Reasons for this could be, for instance, a release from the potential trade-off between extra-pair behaviour and foraging with the low light levels during dawn. Further, low light levels could inhibit efficient mate-guarding, thereby increasing a female's chances for a successful extra-pair copulation. One important factor influencing the timing of extra-pair behaviour may result from the fact that for an extra-pair mating, two individuals from different territories have to meet. The easiest way to meet another individual is to know its position at a given time. This again makes dawn (and, less pronounced, dusk) a perfect candidate time for extra-pair courtships, because (a) the male advertises his location through song (Poesel *et al.* 2004) and (b) the female often sleeps in the nesting cavity (Steinmeyer *et al.* 2010, Perrins 1979 p. 157) and is thus also in a predictable location. Although some studies support the view that the morning period is specifically important for some aspects of extra-pair behaviour in blue tits (Kempenaers 1994a, Poesel *et al. 2004*, Kempenaers *et al.* 2010) and other species (e.g. Double & Cockburn 2000), other extra-pair related behaviours occur throughout the day (e.g. blue tits Kempenaers *et al.* 1992; other species: Currie *et al.* 1998, Pitcher & Stutchbury 2000). Here I investigate whether the time of day influences different types of extra-pair behaviour (Chapter 3, Chapter 4, Chapter 5).

Location. One major question is where extra-pair courtships and copulations are taking place. For instance, in species that do not feed on their breeding grounds, a joint feeding location may be a natural site for meeting an extra-pair partner (Stapleton & Robertson 2006). In species with all-purpose territories such as the blue tit, the encounter of a potential extra-pair mate may require active forays to the extra-pair partner's territory or another location. Such patterns show major variation between species (for a review see Westneat & Stewart 2003), and forays of the different sexes may even serve different functions. In the superb fairy-wren, for example, males visit and court females on their territories throughout the day, whereas females perform covert forays to specific males on their territories during dawn (Double & Cockburn 2000 and references therein). A previous study on blue tits reported that both males and females foray into other bird's territories, although only female foraying behaviour was correlated to extra-pair paternity (Kempenaers *et al.* 1992, Kempenaers *et al.* 1995). In Chapter 4 and Chapter 5, I investigate whether – similarly to the fairy wrens - the location of potential extra-pair interactions varies with the time of the day.

Is the behaviour of one sex more important than that of the other? Since males clearly benefit in evolutionary terms from any extra-pair offspring they produce, while the costs and benefits for females are less clear and may be less pronounced, female extra-pair behaviour may drive relatively

few of the patterns of extra-pair paternity that we observe. However, we already know that at least female extra-territorial forays may influence extra-pair paternity (Kempenaers *et al.* 1992). Therefore, females are clearly not just passive recipients of extra-pair copulations. Indeed, a female's behaviour or state may even be decisive for her partner's extra-pair behaviour, if for instance males change their extra-pair behaviour during their mate's fertile period. I therefore aim to use Chapter 3, Chapter 4, and Chapter 5 to gather more information about the relative importance of male and female behaviour as a driver for the patterns of extra-pair paternity that we observe.

Who mates with whom? Finally, studies focusing on extra-pair behaviour generally ask the question whether a certain type of male or female does or does not have extra-pair offspring. Several parameters have been suggested with mixed support. These are for instance male age, body size, or plumage ornamentation (e.g. Delhey *et al.* 2003, for a review see Akçay & Roughgarden 2007), male song characteristics (pitch, timing, length, or variety; Poesel *et al.* 2006, Chiver *et al.* 2008, Kempenaers *et al.* 2010, Halfwerk *et al.* 2011), and female breeding experience (Whittingham & Dunn 2010). However, extra-pair behaviour is an interaction between two individuals. This problem is usually solved by comparing the social male of a female with her extra-pair male (e.g. Kempenaers *et al.* 1997, Foerster *et al.* 2006, for a review see Akçay & Roughgarden 2007). However, this approach does not take into account the set of other males that the focal female could potentially have had extra-pair offspring with. Here I compare the successful extra-pair sires with all unsuccessful extra-pair males. For such an analysis to be meaningful, the spatial distance between males and females also has to be taken into account, because in most species, extra-pair paternity results mostly from matings with close-by individuals, e.g. neighbours (e.g. Kempenaers *et al.* 1995, Perreault *et al.* 1997, Foerster *et al.* 2003, but see Westneat & Mays 2005, Woolfenden *et al.* 2005, Stewart *et al.* 2006). Using this framework I address in detail the question why a specific female mates outside the pair-bind with a specific male (Chapter 2, Chapter 4).

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Chapter 1

Thiessen polygons as a model for animal territory estimation

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Short communication

Thiessen polygons as a model for animal territory estimation

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Thiessen polygons are often used to model territory characteristics. However, information about the quality of Thiessen polygon-based estimates is currently lacking. We used published data to investigate the match between Thiessen polygons and mapped bird territories regarding territory size, shape and neighbourhood. Although territory sizes and the number of neighbours were strongly correlated between these two methods, both parameters were overestimated by the Thiessen polygons. Therefore, caution is required when Thiessen polygons are used as a model for absolute values and when the assumptions of Thiessen polygons, such as formation of discrete territories and a contiguous study area, are not met.

Keywords: competition, Dirichlet tessellation, focal point, Voronoi diagram.

Because territory acquisition and tenure are important for individual breeding success and survival, measuring territory parameters is often relevant in studies in behavioural ecology. However, mapping territories directly through observations or radiotracking is difficult and time-consuming, and not possible to do *post hoc*. Therefore, an alternative to territory mapping to assess territory boundaries becomes important.

Thiessen polygons (also known as Dirichlet tessellations or Voronoi diagrams) define the area of influence of each focal point (e.g. nest-site) by a polygon encompassing the area closer to the target point than to any other point (Aurenhammer 1991). The definition of Thiessen polygons (henceforth TPs) reflects a competitive process by which the available space (suitable habitat) is partitioned among neighbouring individuals (e.g. Morrell & Kokko 2005). This renders TPs a useful model for estimating territory boundaries and areas, as well as the number and identity of neighbours occupying adjacent territories (e.g. Wilkin *et al.* 2006, Valcu & Kempenaers 2008, Kempenaers *et al.* 2010). TPs are based entirely on focal points, which can be any set of points around which territories are formed ('centres of defence' such as nest-sites: Adams (2001) and references therein). Therefore, territories can be estimated without any further information on the individuals (e.g. their competitive abilities) or the environment (e.g. habitat heterogeneity). This is especially useful for re-analysing existing datasets, where important parameters of the habitat or of the individuals may not be known and cannot be investigated *post hoc*. Significant relationships between territory size estimated by TPs and measures of breeding success indicate that TPs capture biologically meaningful information (e.g. Valcu & Kempenaers 2008, Grabowska-Zhang *et al.* 2012). However, how well TPs approximate the different properties of a territory has not been investigated. On the basis of 14 studies presenting mapped territories, this study investigated the quality of the approximation of the TPs, and potential biases. As possible focal points (e.g. nest-sites) were mostly unknown, we investigated the quality of TPs depending on the distance of the focal points to the centres of mass of the respective mapped territories. We review published literature on the use of TPs and present a framework for the practical application of TPs.

METHODS

We used the digitized mapped territories from 14 published studies on 12 bird species (from nine families) described in Valcu and Kempenaers (2010) and the TPs that were calculated for these 14 studies. As a minor change, we used only the larger of two study areas ('cattle creek') in one study (Wortman-Wunder 1997). We only selected studies in which (1) territories were obtained via detailed observations of territorial behaviour of individually marked animals; (2) territories of more than 10 individuals were mapped; and (3) territories were not obviously constrained by the geography of the study site. All the maps were saved in a raster format and each territory was manually digitized and saved in a vector format (details in Valcu & Kempenaers 2010).

Because studies using mapped territories do not typically provide focal point data (only four studies in our dataset give a focal point position), we used a series of numerical simulations to investigate the influence of the focal points on the construction of the TPs. First, we constructed TPs using the midpoints (centroids, centres of mass) of the mapped territories. Secondly, we defined

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a series of focal points for TP construction by sampling at specific distances from the centroid in random directions. We used distances between 2% (almost identical to the centroid) and 98% (almost on the border of the territory) at 2% intervals. We repeated the random sampling process 50 times for each distance.

Borders of study areas were defined using a convex Ripley–Rasson estimate (Ripley & Rasson 1977). An example is shown in Supporting Information Figure S1. We calculated the territory size and the number of neighbours for both mapped territories and TPs. The number of neighbours was defined as the number of individuals with adjoining territory borders for the TPs, and the number of territories within a distance defined such that each individual had at least one neighbour for the mapped territories. We used linear mixed-effects models with 'study' as the random intercept, the mapped territory measure as the dependent variable and the corresponding TP-based estimate as the predictor. By overlaying mapped territories and TPs we were able to calculate the underestimation and the overestimation of the polygons for both territory location and neighbour identity. We defined 'underestimation' as the percentage of the mapped area (or neighbours) that was not assigned to the corresponding TPs, and 'overestimation' as the percentage of the TP area (or neighbours) that exceeded (or projected beyond) the corresponding mapped area (or neighbours).

We used R 2.14.1 for all statistical analyses (R Development Core Team 2011). Specifically, we used the packages 'lme4' for linear mixed-effect models (Bates *et al.* 2011), 'effects' for graphical representation of the regressions (Fox 2003), 'spatstat', 'maptools', 'spdep', 'rgeos' and 'rgdal' for spatial calculations, and 'stats' for fitting smooth lines (Baddeley & Turner 2005, Bivand *et al.* 2008, R Development Core Team 2011, Bivand & Rundel 2012, Keitt *et al.* 2012, Lewin-Koh & Bivand 2012, respectively). TPs were calculated following the method described in Valcu and Kempenaers (2010). Estimates of territory size were log-transformed to achieve normality and scaled (standardized and centralized) to achieve valid intercepts and comparability among studies. The number of neighbours was centralized to achieve valid intercepts. All transformations were performed for each study using all data points for the respective area (values of both TPs and mapped territories) to maintain comparability of the absolute values of the two methods. Note that *t*-values and *P*-values reflect effects caused by both the true correlations and the spatial autocorrelations among the TPs (Valcu & Kempenaers 2010).

RESULTS

The mean (± se) number of mapped territories per study was 30.2 ± 6.4 (range 13–107). Territory size as estimated by the TPs was strongly correlated with mapped territory size, although it was on average overestimated (Fig. 1a, slope = 0.87 ± 0.06, $t = 15.30$, $P < 0.001$, $n = 423$; intercept = −0.07 ± 0.01, $t = -6.05$, $P < 0.01$). Similarly, the number of neighbours estimated by the TPs was strongly correlated with the number of neighbours defined by the mapped territories, although the number of neighbours was overestimated on average (Fig. 1b, slope = 0.56 ± 0.04, $t = 13.02$, $P < 0.001$, $n = 423$; intercept = −1.31 ± 0.15, $t = -8.95$, $P < 0.01$). All parameters decreased in fit as the distance of the focal points used for TP construction to the centroid of the mapped territory increased. However, both regressions (territory size and number of neighbours) remained significant throughout (all *P*-values ≤ 0.01).

The mapped territories of the 14 studies showed little overlap (range 0–0.8%). The mean percentage of the mapped area that was not assigned to the corresponding TP ranged from 2% to 25% (11 ± 2%, Supporting Information Fig. S2a, 'underestimation'), and the mean percentage of the area of the TP that exceeded the corresponding mapped area ranged from 26% to 72% (49 ± 4%, Fig. S2a, 'overestimation'). The mean percentage of neighbours that were not identified by the TP ranged from 0% to 16% (4 ± 1%, Fig. S2b, 'underestimation'), and the mean percentage of neighbours identified by the TP that did not correspond to neighbours based on the mapped area varied between 16 and 44% (30 ± 2%, Fig. S2b, 'overestimation'). Neighbour identity and territory location showed a decreasing fit as the distance of the focal point used for Thiessen polygon construction to the centroid of the mapped territory increased (Fig. S2).

We defined a two-step framework for the use of TPs in a specific study where focal points could be assessed (Fig. 2). At step 1, the habitat or the population boundary is selected assuming that all space within that boundary is partitioned among individuals. In some cases, clear boundaries can be defined *a priori* (e.g. an island in a lake, a forest patch surrounded by agricultural fields). When the area that encompasses a population is not clear *a priori* but relatively well defined, then the boundary can be estimated as a convex polygon. If any boundary can be established, TPs can be constrained to lie within the boundary. Otherwise edge territories should be removed and/or polygon boundaries should be constrained by intersection with a circle of a given radius to avoid unrealistically large territories. These methods can be combined in cases in which boundaries can only be established for some parts of the study area (e.g. a lake at one side of the study forest). At this point, TPs completely cover the area within which they are calculated. At step 2, if *a priori* knowledge of covariation of individual traits with territory parameters exists, TPs

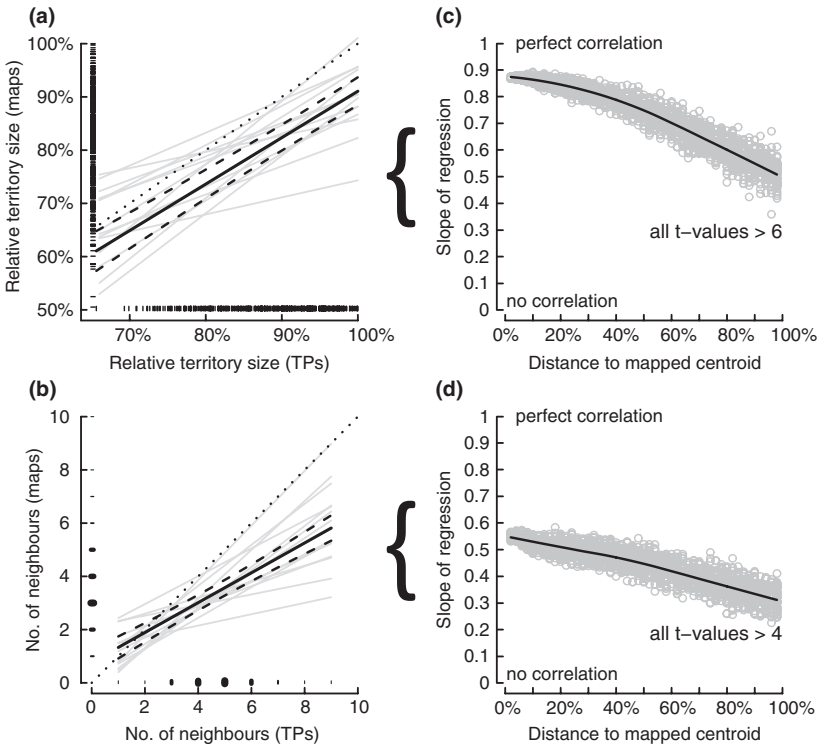

Figure 1. Regression of mapped territories against Thiessen polygons with respect to territory size (a, c) and the number of neighbours (b, d). Panels (a) and (b) show model estimates and confidence intervals (black), regressions for individual studies (grey) and the data frequency (parallel to the axes). The dotted line indicates a perfect fit. Axes in (a) are given as the percentage of the largest (log-transformed) value per study. Note that in contrast to the statistical model, the data shown here are non-centralized. Panels (c) and (d) show the slope of the respective regression depending on the distance of the focal point used for the construction of Thiessen polygons to the centroid of the mapped territory. Grey circles are individual estimates from the randomizations, and the black line is a smooth curve through the randomized points.

can be further improved by scaling the polygon through a homothetic transformation (e.g. younger males' territories are smaller than those of older males, and are therefore down-scaled) or by altering its shape in accordance with a known behavioural process (e.g. the competitive ability of two experienced breeders may be more similar than that of an experienced and a first-time breeder, so that in the latter case the territory border can be moved in favour of the experienced breeder).

DISCUSSION

We assessed how well TPs estimated the four most commonly used parameters of territories (size, location, number of neighbours and neighbour identity). There was a strong correlation between mapped territories and the estimates from TPs regarding territory size and number of neighbours (Fig. 1). Both territory size and the number of neighbours were overestimated by the TPs. This overestimation is also obvious in Figure S2, and has to be kept in mind when estimating absolute territory sizes from TPs (see below). Note that Figure 1(b) suggests a stronger overestimation when more neighbours are present. With regard to territory size and the number of neighbours, the strength of the correlation between TPs and mapped territories decreased when the focal points used for the construction of TPs were further away from the centroids of the mapped territories, but remained strongly significant at all distances.

Although the mean percentage of the mapped area that was not assigned to the corresponding TP ('underestimation') was small, the mean percentage of the area of the TP that did not overlay the corresponding mapped area ('overestimation') was large. For example, for more than half of the territories, 46% of the area that was predicted to be defended as a territory by the TPs did not overlay the corresponding mapped territory. Similarly, the identities of almost all mapped neighbours were correctly predicted, whereas for half of the territories more than 25% of the TP neighbours were mis-assigned (Fig. S2). TPs are therefore expected to be a 'noisy' measurement for studies on, for example, environmental attributes of territories. They should not be used when an overestimation of territory location or neighbour

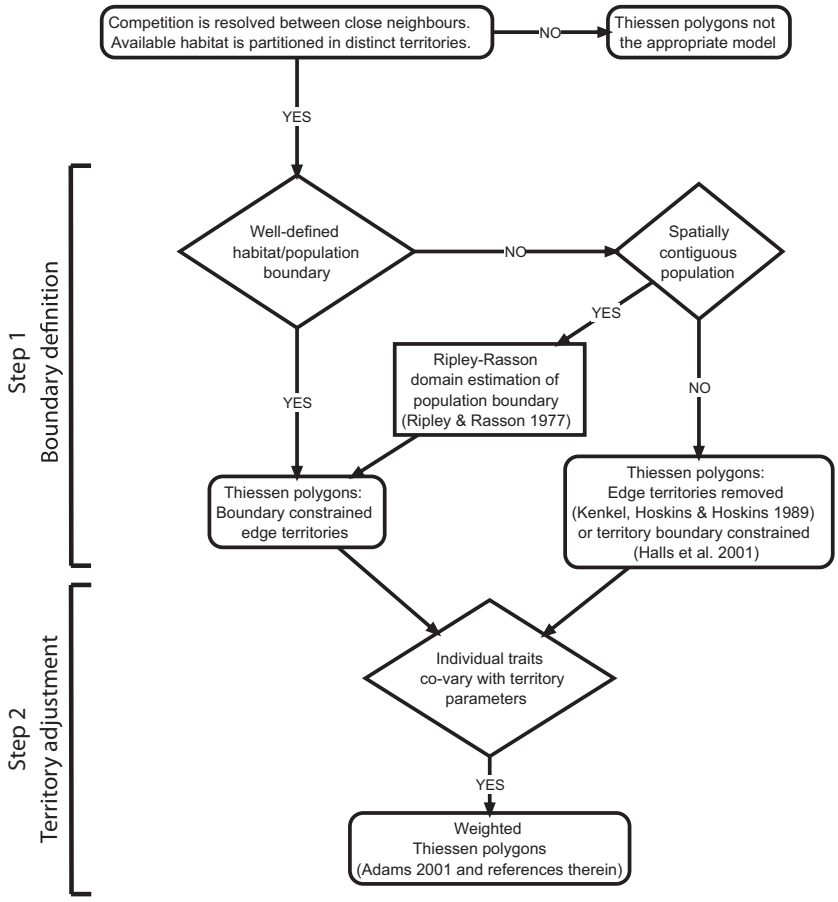

Figure 2. Flow chart for the application of Thiessen polygons to estimate territory characteristics.

identity can lead to spurious results. Both overestimation and underestimation increased if focal points were fixed to be further away from the centroid of the mapped territory (Fig. S2).

TPs are an appropriate model for classically territorial species (Adams 2001). As expected for territorial species, the mapped territories overlapped little in the focal 14 studies (range 0–0.8%). This means that discrete territories were defended. However, some studies had low-density settlement. Additionally, boundaries of study areas and unusable habitat patches were rarely defined. This is in part responsible for both the overestimations of territory size and the number of neighbours. To avoid such biases and increase the fit of TPs, it is important to consider carefully how to set biologically meaningful conditions for a specific context (Fig. 2). The construction of TPs resembles the biological process of territory formation only if the available habitat is partitioned into distinct territories through a competitive process between adjacent individuals. If territories are formed by fundamentally different mechanisms (e.g. habitat patchiness, distance of movement from the nesting site), there is no theoretical basis for the use of TPs. In low-density populations, where neighbour encounters may be only partly responsible for territory formation, unrealistically large territories can be avoided by assuming that an individual will not move further than a certain distance from its nesting site (McLeod *et al.* 2002). However, in many cases the radius of the respective circle will not be clear *a priori* (e.g. Wilkin *et al.* 2006). The information that will be included to achieve a valid fit of the TPs largely depends on the available information on habitat structure, study area boundaries and individual parameters (Fig. 2), as well as on the precision of the parameter estimate needed for addressing the respective research question.

In conclusion, our results suggest that TPs without any biological or ecological refinements are a useful tool for estimating territory areas, locations and neighbourhoods of territorial bird species. However, they generally tend to overestimate both the territory size and the number of neighbours. They may be fine-tuned by including ecological and biological information in the construction process, which in turn may decrease

overestimations. If the main assumptions underlying the use of TPs are not met, or if the overestimation of parameters is problematic for the specific study, other models should be used that are less general and carefully adjusted to the specific context.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Visualization of mapped territories (grey) vs. Thiessen polygons (black).

Figure S2. Underestimation and overestimation of the Thiessen polygons regarding (a) territory area and (b) neighbour identity in relation to the distance of the focal point to the respective centroid.

Chapter 2

Spatial patterns of extra-pair paternity: beyond paternity gains and losses

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Lay summary

Birds of many species form a socially monogamous pair bond, but often mate with other individuals. These so-called extra-pair copulations are the result of a behavioural interaction between at least two individuals: the male and the female that copulate. Nevertheless, extra-pair behaviour is usually investigated either from the male or from the female perspective only. The questions that are addressed are thus usually limited to why certain individuals do or do not have extra-pair offspring. Whether an individual has extra-pair offspring, however, may also depend on the quality of potential extra-pair mates in the surroundings. The social setting that an individual experiences may therefore have a large impact on whether this individual will or will not have extra-pair offspring. When investigating extra-pair behaviour, or indeed any other behaviour that involves interactions between two individuals, it is therefore important to shift the focus of analysis from the individual to both interacting individuals, here the male and the female that do or do not have extra-pair offspring with each other. In this paper, we present an approach which can be used to investigate why a pair of individuals has extra-pair offspring, and that takes into account the characteristics of the focal male and the focal female, as well as of the social and natural environment that surrounds them. To demonstrate this approach, we apply it to a dataset on extra-pair paternity in blue tits from two populations, spanning a total of twelve breeding seasons.

Summary

1. Most studies on extra-pair paternity (EPP) focus either on a specific male's extra-pair gains or his extra-pair losses. For an individual bird however, mate choice or mate availability may underlie strong spatial restrictions. Disregarding this spatial aspect may underestimate or mask effects of parameters influencing observed EPP patterns.

2. Here, we propose a spatially explicit model for investigating the probability of having extra-pair offspring (EPO) within local networks of breeding pairs. The dataset includes all realized and unrealized potential extra-pair matings. This method is biologically meaningful because it allows (a) considering both members of an extra-pair mating as well as their social mates, and (b) direct modelling of the spatial context in which extra-pair behaviour occurs. The method has the advantage that it can provide inference about the relative contribution of spatial and non-spatial parameters, and about the relative importance of male and female neighbourhoods.

3. We apply this method to parentage data from 1025 broods collected over 12 breeding seasons in two independent study populations of blue tits (*Cyanistes caeruleus*). We investigate a set of predictions based on the EPP literature, namely that EPP depends on male age and body size, breeding density, and breeding synchrony. In all analyses, we

control for breeding distance, a parameter that is expected to influence EPP even under random mating.

4. The results show that older and larger males were more likely to sire EPO, but both effects decreased with increasing breeding distance. Local breeding density but not synchrony predicted whether a particular male-female combination had EPO, at least in one of the study areas. Apart from breeding distance, male age had the strongest effect on EPP, followed by a measure of breeding density. The method thus allows a comprehensive assessment of the relative importance of different types of spatial and non-spatial parameters to explain variation in the occurrence of EPP, while controlling for the fact that individuals that breed further apart are less likely to have EPO.

5. The proposed approach is not limited to investigate EPP, but can be applied to other behavioural interactions between two individuals, such as dominance, competition, and (social) mating.

Key-words: competition, extra-pair behaviour, female behaviour, promiscuity, male behaviour, mate choice, mating system, neighbourhood, sexual selection, social network

Introduction

Sexual behaviour outside the social pair-bond occurs frequently in socially monogamous birds (reviewed by Griffith, Owens & Thurman 2002). In passerines, extra-pair paternity (EPP) has been reported in 86% of the studied species (Griffith, Owens & Thurman 2002). However, levels of EPP may vary dramatically between species, between populations of the same species, and between individuals within populations (Petrie & Kempenaers 1998, Griffith, Owens & Thurman 2002, West neat & Mays 2005).

Extra-pair mating patterns may be influenced by the behaviour of at least four parties: a potential extra-pair female and her social mate, and a potential extra-pair male and his mate (Petrie & Kempenaers 1998, Westneat & Stewart 2003, Canal, Jovani & Potti 2012). However, most studies investigating patterns of extra-pair mating focused on either male or female extra-pair behaviour. This fails to provide a 'balanced perspective of the sexes' (Westneat & Stewart 2003) and may not reflect the biological mechanisms underlying the observed extra-pair mating patterns. Similarly, most studies investigate male EPP gains and losses, and correlate these either with characteristics of the focal males (e.g. body size, Kempenaers, Verheyen & Dhondt 1997) or with ecological parameters (e.g. breeding density, Westneat & Sherman 1997). However, the spatial setting in which extra-pair behaviour occurs is rarely modelled explicitly (e.g. Westneat & Mays 2003).

In blue tits *Cyanistes caeruleus L.* (Kempenaers *et al.* 1992, this study) and other species (e.g. *Eudocimus albus* (white ibis): Frederick 1987, *Dolichonyx oryzivorus* (bobolink): Bollinger & Gavin 1991, *Tachycineta bicolor* (tree swallows): Kempenaers *et al.* 1999, *Acrocephalus sechellensis* (Seychelles warbler): Komdeur 2001, *Passerculus sandwichensis* (savannah sparrows): Freeman-Gallant *et al.* 2005) the majority of extra-pair matings occur between individuals that breed nearby, and often they are direct neighbours (but see *Agelaius phoeniceus* (red-winged blackbird): Westneat & Mays 2005). Thus, disregarding the spatial component that limits extra-pair behaviour may underestimate or obscure the effect of parameters that influence extra-pair mate choice and mate competition. This is the case even in small populations, as long as the spatial scale at which extra-pair mating takes place is considerably smaller than the population itself. The conventional way of investigating patterns of extra-pair mating has another important drawback: it is generally not possible to model spatial parameters (e.g. breeding synchrony or breeding distance) and non-spatial parameters (e.g. age, body size or experience) within the same model.

Here, we propose a spatially explicit method for investigating the probability of having extra-pair offspring (EPO) within local networks of breeding pairs. The approach considers all male-female combinations that can potentially occur (except for the social pair) as potential extra-pair partners. Instead of investigating which parameters influence the probability that an individual bird has EPO, we examine which parameters influence the likelihood that a particular male-female "extra-pair" has produced EPO taking into account characteristics of all potential male-female combinations that breed at the same distance (same neighbourhood). This method follows more closely the biological arena in which extra-pair matings take place, and it allows considering both members involved in an extra-pair mating as well as their social mates. Additionally, the method allows to explicitly include information about those male-female combinations that did not have EPO with each other. Moreover, it allows direct modelling of the spatial context in which extra-pair behaviour occurs, and provides inference about the relative contribution of spatial and non-spatial parameters. The aim of this paper is to describe the general approach and illustrate it with a case study.

General Approach (Fig. 1, Fig. 2)

EPP has two basic behavioural requirements which usually remain unobserved. (a) A male and a female that do not form a social pair have to meet. Whether they meet depends on the overlap of the individuals' spatio-temporal movement patterns. (b) The male and the female have to copulate. Males are generally assumed to utilize any mating opportunity, whereas females may be choosy, and may mate selectively with some males but not with others.

Thus, the male and the female behaviour, interactions with their environment, their social mates and potential competitors, and post-copulatory processes determine which male and which female in a population have EPO with one another. Whether an individual has EPO may therefore strongly depend on the behaviour of the potential extra-pair partners (e.g. a low-quality male may be rejected by all females). Based on parentage data, we can now ask why specific male-female combinations

have EPO, whereas others do not.

To make inferences about why specific pairs have EPO, we use a generalized linear mixed-effect model (GLMM), where we consider all potential "extra"-pairs (i.e., all male-female combinations in the population apart from the social pair, including individuals without any EPO) as individual data points (Fig. 1, Box A). We then add information on which of these male-female combinations actually had EPO or not (Fig. 1, Box B), which is the response variable in the model. For every male-female combination we can then add attributes which may influence the likelihood for this pair to have EPO. Such parameters may include traits of the focal individuals, the distance between the focal individuals' territories, traits of their social mates, or traits related to their local neighbourhood (Fig. 1, Box C). We defined the 'neighbourhood' as all individuals that breed at a fixed distance (in territories) from the focal individual. These attributes are the explanatory variables in the model. Which explanatory variables are included will depend on the available information, the specific hypotheses one wants to test, and the sample size. Alternative models can be constructed and compared, for example, those that consider male or female traits in absolute terms (population-wide comparison) with those that consider male or female traits relative to other males or females in the local neighbourhood (spatially explicit model).

Our approach shows similarities to "social network analysis" (e.g. Aplin *et al.* 2013, McDonald 2013), but there are some key differences. Studies investigating social networks are based on interactions among individuals, whereby individuals are defined as 'nodes' and interactions as 'edges'. Social network analyses generally focus on two aspects. (a) The 'nodes' of the network and their properties: individuals and their positioning within the network (e.g. centrality or connectedness) are investigated, often in relation to other traits of the individual (e.g. Aplin *et al.* 2013). (b) The grouping structure of 'nodes' in the network. This approach is used to investigate differences between groups of individuals that e.g. interact more often (e.g. Aplin *et al.* 2013), or to investigate attributes or behaviours of individuals given the group they live in (McDonald *et al.* 2013). Network analysis thus generally asks questions about the properties or grouping structure of individuals in the network, but usually does not investigate what determines the strength of the association between two individuals. A few studies investigated correlations between strengths of different associations, for instance whether the number of social interactions of a pair of individuals predicts their future pairing status (e.g. Kurvers *et al.* 2013, Henry *et al.* 2013, McDonald 2009). These studies are limited, however, to correlations among two variables, as the statistical approaches only allow investigating the correlation between two interaction matrices. This constraint is lifted in our approach; analysing the relationship between dyadic combinations of focal individuals (here male-female combinations) as the response variable in a generalized linear mixed-effect model gives us the opportunity to investigate correlations with multiple variables (adjusted to their spatial scale) and their interactions.

Why should one use this approach and focus on all possible male-female combinations (all potential extra-pair partners), instead of using conventional methods of analysis? The advantage of our approach is that it allows testing hypotheses about mating decisions of a pair of individuals based on the specific social and ecological environment of each of the individuals, explicitly including traits of all

A all ♂-♀ combinations		B EPP	C potential correlates EPP							
♂ ID	♀ ID	yes / no	distance class	♂ traits	♀ traits	traits of ♂'s social mate	traits of ♀'s social mate	traits of ♀ relative to other ♀♀ in the ♂'s neighbourhood	traits of ♂ relative to other ♂♂ in the ♀'s neighbourhood	...
$♂_1$	$♀_2$	No	2	$♂_1$ traits	$♀_2$ traits	$♀_1$ traits	$♂_2$ traits	$♀_2$ traits - mean(traits of ♀♀ around $♂_1$)	$♂_1$ traits - mean(traits of ♂♂ around $♀_2$)	...
$♂_1$	$♀_3$	No	3	$♂_1$ traits	$♀_3$ traits	$♀_1$ traits	$♂_3$ traits	$♀_3$ traits - mean(traits of ♀♀ around $♂_1$)	$♂_1$ traits - mean(traits of ♂♂ around $♀_3$)	...
...	...	...	...	...	...	...	...	...	...	...
$♂_2$	$♀_1$	No	2	$♂_2$ traits	$♀_1$ traits	$♀_2$ traits	$♂_1$ traits	$♀_1$ traits - mean(traits of ♀♀ around $♂_2$)	$♂_2$ traits - mean(traits of ♂♂ around $♀_1$)	...
$♂_2$	$♀_3$	Yes	1	$♂_2$ traits	$♀_3$ traits	$♀_2$ traits	$♂_3$ traits	$♀_3$ traits - mean(traits of ♀♀ around $♂_2$)	$♂_2$ traits - mean(traits of ♂♂ around $♀_3$)	...
...	...	...	...	...	...	...	...	...	...	...
data points (unique ♂-♀ combinations) random factors (♂ identity, ♀ identity, ...)		response variable	explanatory variables							

Figure 1. Illustration of the general modelling approach. All unique male-female combinations, excluding the social pair, constitute the individual data points (Box A). All individuals are therefore represented multiple times in the dataset, and individual IDs need to be included as random factors. Provided that all adults and offspring have been sampled, parentage analysis allows assigning each male-female combination to one of two categories: having EPO together in a given season or not. This is the binomial response variable (Box B). Parameters that may explain variation in the observed patterns of EPP can now be added to the model (Box C). These can include (a) an estimate of the geographic distance between the focal male and female (e.g. distance between their respective nests), (b) traits of the focal male and female, either in absolute terms or relative to other males and females in the neighbourhood, (c) traits of the pair (e.g. whether they have been observed interacting), and (d) traits of the social mate of the male and female, either in absolute terms or relative to other mates in the neighbourhood.

other available potential extra-pair mates (independent of their extra-pair success). This can give us a better understanding of the causalities underlying EPP and about potential mate choice decisions. A simple example illustrates the issue. A researcher may want to investigate whether males that father EPO with neighbouring females differ from males that father EPO at larger distances. The researcher may find that extra-pair sires that have a territory far away, but not neighbouring extra-pair sires, are less related to the focal female (in absolute terms or compared to her social mate). Assuming by default a population with a spatially homogeneous genetic structure, the researcher could conclude that while some females seem to perform extra-pair copulations with random neighbours (e.g. due to male harassment), other females actively roam around to seek extra-pair copulations with unrelated males to increase the heterozygosity of their offspring (indicating female choice). However, relatedness among neighbours may on average be higher than among non-neighbours (due to small-scale genetic population structure as reported for blue tits; Foerster *et al.* 2006), such that extra-pair mates that breed further apart will always be less related (see also Foerster *et al.* 2003). Thus, without information about local genetic structure, the alternative hypothesis that all females behave in the same passive manner (e.g. copulate with an extra-pair male to avoid further harassment) is equally plausible. The two hypotheses are difficult to disentangle with conventional methods, because males that do not have EPO cannot be taken into account. With the approach presented here, we can investigate whether females have EPO with less related males *given all males available within the same area as the successful extra-pair sire*, and whether this effect differs depending on breeding distance (e.g. direct neighbours, second-rank neighbours …). This allows us to tease apart correlations that arise due to the underlying spatial structure of the data (e.g. genetic relatedness among individuals depends on distance), and correlations that arise through other

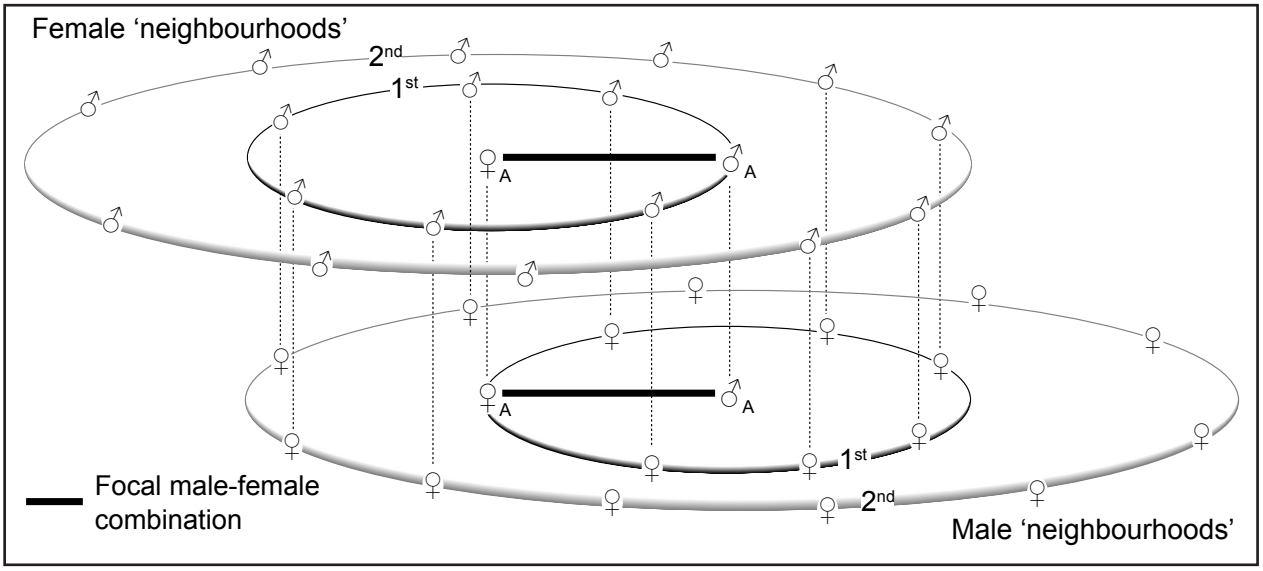

Figure 2. Visualization of a focal male-female combination and the respective neighbourhoods. The focal male-female combination is represented by male A and female A, which are connected by a black bar. The upper two circles centre on female A (the focal female) and represent the focal female's first and second order neighbours. The lower two circles centre on male A (the focal male) and likewise represent the focal male's first and second order neighbours. Dotted lines indicate that the connected individuals belong to the same territory (identical birds or social pair members). The focal male and the focal female may or may not have EPO with each other, and this may depend on their own characteristics, and on characteristics of the respective neighbourhoods. The female "neighbourhoods" refer to all the males surrounding the focal female A that are potential extra-pair mates. Similarly, the male "neighbourhoods" refers to all females surrounding the focal male A that are potential extra-pair mates. Neighbourhoods are defined as 1st, 2nd, etc… order depending on the distance (number of territories) from the focal bird.

mechanisms, such as active mate choice (e.g. for less related males). Because interactive behaviours (such as mating, aggression, and cooperation) usually take place on a local scale, this approach may prove useful in a wide array of contexts (see Discussion).

Case study

We apply the proposed method to a data set obtained through parentage analyses of 1025 broods of blue tits from two populations breeding 400 km apart, encompassing a total of 12 seasons. This allows us to compare the results of these independent studies of the same species. We first control for the breeding distance among two individuals, because EPP mostly occurs between close neighbours (e.g. Kempenaers *et al.* 1992, Kempenaers *et al.* 1999, Freeman-Gallant *et al.* 2005, Canal, Jovani & Potti 2012). This is expected even under random mating, as long as neighbouring individuals have a higher chance to meet. To avoid an over-parameterized model and to demonstrate the validity of this approach in comparison with previous approaches, we focus on a limited set of explanatory variables that are usually available in this type of studies and are known to affect EPP. These are male age and body size (e.g. Akçay & Roughgarden 2007 and references therein), breeding density (e.g. Westneat & Sherman 1997, Thusius *et al.* 2001, but see Stewart *et al.* 2006), and breeding synchrony (e.g. Yezerinac & Weatherhead 1997, Thusius *et al.* 2001, Canal *et al.* 2012, but see Kempenaers 1997, Stewart *et al.* 2006). Additionally, we explicitly included the interaction between breeding distance and male body size and age, because a previous study on blue tits suggested that different mechanisms may be driving EPP for close vs. distant individuals (Foerster *et al.* 2003). To clarify the hypotheses addressed in this study, we stated the underlying scientific questions for each explanatory variable in Table 1. The method can easily be extended to other parameters, such as characteristics of the female's social mate (e.g. body size: Neto, Hansson & Hasselquist 2010, Yezerinac & Weatherhead 1997, plumage colour: Delhey *et al.* 2003, or behaviour: Kempenaers, Verheyen & Dhondt 1997), or to characteristics of the focal male-female combination (e.g. relatedness: Akçay & Roughgarden 2007, Kempenaers 2007).

Materials and methods

Terminology

Each data point consists of a focal male and a focal female which could potentially have EPO together (see below, Fig. 2). We call this the 'focal male-female combination'. Because a focal male-female combination represents potential extra-pair partners, it cannot by definition be a social pair.

Table 1. Overview of variables initially considered for inclusion in the model to explain variation in the occurrence of EPP in two blue tit populations. For each variable, we provide a definition, indicate how the mean used for centring was calculated and explain the specific question (hypothesis) that can be tested using this approach. Variables that were excluded from the model because of strong collinearities are marked in italic. Note that all means were calculated within year (breeding season).

Variable	Definition of raw variable	Female traits and surroundings		Male traits and surroundings	
		Mean used for centring	Scientific question	Mean used for centring	Scientific question
Breeding distance	Distance between two birds in number of territories; identical for focal ♂s and ♀s; defines the 'neighbourhood' of an individual at a specific breeding distance	Not centred	Allows controlling for the fact that EPO are more likely among close-by individuals.	Not centred	Allows controlling for the fact that EPO are more likely among close-by individuals.
♂ age	Yearling (1st year, coded as '0') or adult (older, '1')	*Mean at the respective breeding distance*	*Is the potential extra-pair ♂ older compared to the other ♂s at the same distance?*	Not applicable (two-level factor)	Are adult ♂s in the population more likely to sire EPO than yearlings?
♂ body size	Tarsus length of the focal ♂ in mm	*Mean at the respective breeding distance*	*Is the potential extra-pair ♂ larger compared to the other ♂s at the same distance?*	Population mean	Are large ♂s in the population more likely to sire EPO than smaller males?
Territory size	Estimated area of a territory in m^2 [1)]	Population mean	Are ♀ living in large territories more/less likely to be involved in extra-pair behaviour?	Population mean	Are ♂s living in large territories more/less likely to be involved in extra-pair behaviour?
Number of neighbours	Number of neighbours at a specific distance class (e.g. number of direct or 1st order neighbours)	Mean for each breeding distance class within the population (e.g. mean number of 1st order neighbours of all birds in the population) [2)]	Does the proportion of realized EPP events of a ♀ depend on the number of available mates (at the same distance class)?	Mean for each breeding distance class within the population (e.g. mean number of 1st order neighbours of all birds in the population) [2)]	Does the proportion of realized EPP events of a ♂ depend on the number of available mates (at the same distance class)?
Relative breeding asynchrony	Difference in the start of laying between the focal ♀ and the female of a (neighbouring) ♂ in days	Mean at the respective breeding distance	Is a ♀ more/less likely to be involved in extra-pair behaviour with males paired to asynchronously breeding ♀s in her surroundings?	Mean at the respective breeding distance	Is a ♂ more/less likely to sire EPO with asynchronously breeding ♀s in his surroundings?

[1)] The square-root of the raw values was used to account for the two-dimensional nature of the variable.

[2)] Transformation at breeding distance class level is necessary to take into account that the mean number of neighbours intrinsically increases with increasing distance class.

The study sites

We use data from two study areas which contained the same type of nest boxes (inside dimensions: 9 x 12 cm; entrance hole diameter: 26 mm; distance to nest box floor: 16 cm). The first area is a mixed-deciduous woodland close to Vienna, Austria ('Kolbeterberg'; 48° 13' N, 16° 20' E, ca. 50 ha, ca. 3.5 boxes / ha), which was studied between 1998 and 2004. The second study area is in a mixed-deciduous oak forest close to Landsberg am Lech, Germany ('Westerholz', 48°08' N 10°53' E, ca. 40 ha, 7 boxes / ha), which was studied between 2007 and 2011. For more details regarding the study areas and the general field procedures, see Foerster *et al.* 2003 and Delhey *et al.* 2007 (Kolbeterberg) and Schlicht *et al.* 2012 (Westerholz).

Data on extra-pair paternity

We took blood samples (approx. 50 µl) for parentage analyses from all breeding adults (captured 8 – 11 days after the first egg hatched) and from all nestlings (when the oldest nestling in a brood reached the age of 14 days). We also collected dead nestlings and unhatched eggs and genotyped all if sufficient quality DNA could be extracted. Some nestlings disappeared from the nest box at an early age and were not sampled. These young are expected to be the less developed nestlings from a brood, and were therefore presumably late in both hatching and laying order. Because EPO are more often found among the first laid eggs in blue tit clutches (Magrath *et al.* 2009), we expect that only few broods were erroneously assigned to the "no EPO" category. Because parentage analysis was done using a panel of 8-11 highly informative microsatellite markers (on average 25 alleles per marker), and because the biological mother is usually known with certainty (no intraspecific brood parasitism), the probability of false exclusion of the social male and the probabilities of false inclusion of an extra-pair male are low (~$3*10^{-7}$ and on average ~10^{-5}, respectively). Moreover, we only assigned an offspring to an extra-pair male when this male was the only candidate assigned with high confidence and with 0-1 mismatches, or when the same male had already sired at least one other offspring in the same brood with high confidence. For further details see Foerster *et al.* 2003 and Delhey *et al.* 2007 (Kolbeterberg), and Schlicht *et al.* 2012 (Westerholz).

It is important to keep in mind that the patterns of EPP we detected are not identical to and may underestimate the actual extra-pair mating patterns in the population. Because the latter data cannot currently be obtained, we assume that the occurrence of EPO in a brood reflects the extra-pair copulation patterns, at least in the sense that a higher rate of extra-pair copulations increases the likelihood of an extra-pair fertilization (as proposed by Brommer *et al.* 2007).

Territory mapping

We estimated the spatial distribution of territories within each breeding season using Thiessen polygons (Valcu & Kempenaers 2010). This method partitions the space among the individuals by assigning each point within an area to the closest point of interest (in this case an occupied nest box). This approach reflects the positions of breeding territories at relatively high densities (as in our study), because territories form as individuals partition the available space among them (Schlicht, Valcu & Kempenaers 2014). The approach also allowed us to define territory size and neighbour identities for all individuals. The 'neighbourhood' generally refers to all individuals breeding at the same distance as the focal male-female combination (see below) and is either centred around the female or around the male of the focal male-female combination. This means that when the focal male-female combination consists of direct (second rank …) neighbours, we calculate variables in relation to all direct (second rank …) neighbours of the female (= 'female neighbourhood') or in relation to all direct (second rank, …) neighbours of the male (= 'male neighbourhood). This allows us to investigate why a specific male-female combination – given their breeding distance – had EPO with one another.

Breeding parameters

We defined 'breeding distance' as the distance – in number of territories – between the focal male and female. Direct neighbours thus have a breeding distance of '1'.

Local breeding density may be expressed as the number of breeding pairs per unit area, but it is also reflected by the number of neighbours (e.g. Sundberg & Dixon 1996, Gray 1996, Perreault, Lemon & Kuhnlein 1997, Chuang, Webster & Holmes 1999). If extra-pair mating depends on the availability of potential mates, then individuals with many neighbours should have EPO with more individuals. Conventionally, the null hypothesis is thus that an increasing number of neighbours does not increase the number of EPP events for an individual. At least at very low densities, one would expect this hypothesis to always be rejected, because EPP by definition can only occur when mates other than the social mate are available. By including each individual as many times as there are potential partners we already correct the number of EPP events for the number of available mates. In this context the null hypothesis is thus that the proportion of realized (vs. available) EPP events is constant with an increasing number of neighbours. This is equivalent to assuming that the number of available mates and the number of EPP events changes proportionately. Three different scenarios arise from this null hypothesis (Fig. 3). (a) No effect of the number of neighbours on the proportion of realized EPP. This indicates that the null hypothesis cannot be rejected, and suggests that the number of EPP events of an individual strongly depends on the availability of mates (e.g. Formosa & Tüttle 2009, Dunn *et al.* 1994, Stewart *et al.* 2006). (b) A negative effect of the number of neighbours on the proportion of realized EPP, suggesting a decreasing proportion of realized EPP events with an increasing number of neighbours. This may indicate that the number of an individual's EP mates is limited or (as an extreme case) even fixed. Such a scenario may occur, for instance, if

more neighbours are not directly linked to encounters with more individuals, or if courtships and/or copulations are costly (e.g. Komdeur 2001, Dunn *et al.* 1994). (c) A positive effect of the number of neighbours on the proportion of realized EPP, suggesting an increasing proportion of realized EPP events with an increasing number of neighbours. This could occur, for instance, if an increase in the number of neighbours increases the chances that at least one of them is suitable for EPP (e.g. Shellman-Reeve & Reeve 2000, Johnsen & Lifjeld 2003).

As measures of breeding density, we estimated territory size and the number of neighbours for all individuals based on Thiessen polygons. Although the number of neighbours is calculated on a local scale (see below), we standardize the number of neighbours using population means. We are therefore investigating population-wide effects of breeding density. We used the square-root of territory size to correct for the two-dimensional nature of the data. We assessed the number of neighbours at the breeding distance of the focal male-female combination. Thus, if the focal male and the focal female are direct (second-rank, or third-rank) neighbours, their number of direct (second-rank, and third-rank, respectively) neighbours are assessed. Since the number of neighbours intrinsically differs among different breeding distances (Fig. 4), we then subtracted the mean number of neighbours found at the respective breeding distance throughout the population (Table 1).

Breeding synchrony is often defined based on the overlap in fertile period of females breeding within a certain area in a given season (e.g. Kempenaers 1993, Johnsen & Lifjeld 2003, Westneat & Mays 2005, Stewart *et al*. 2006). To obtain a variable with a similar biological meaning for individual male-female combinations, we here define 'breeding asynchrony' as the absolute value of the difference in first egg date between the focal female and the focal male's mate (Fig. 5). In the model we used only relative breeding asynchrony, calculated once from the focal male's perspective (by subtracting the mean breeding asynchrony with all other potential extra-pair females surrounding the male from the breeding asynchrony of the focal male-female combination; 'male asynchrony'), once from the focal female's perspective (by subtracting the mean breeding asynchrony with all other potential extra-pair males surrounding the female from the breeding asynchrony of the focal male-female combination; 'female asynchrony'; Table 1).

We compared characteristics of the two study populations (Table S1). The two populations did not differ in the number of breeding pairs, in breeding asynchrony among potential extra-pair partners, and in the percentage of adult (older) compared to yearling males (first year breeders). However, in the study area Kolbeterberg territories were on average larger (i.e., lower breeding density), males were larger, and females started to lay significantly earlier, laid larger clutches, hatched more offspring and were more likely to have EPO than in Westerholz. In Westerholz, almost all extra-pair fathers were known breeding males, and therefore paternity assignment was more complete than in Kolbeterberg.

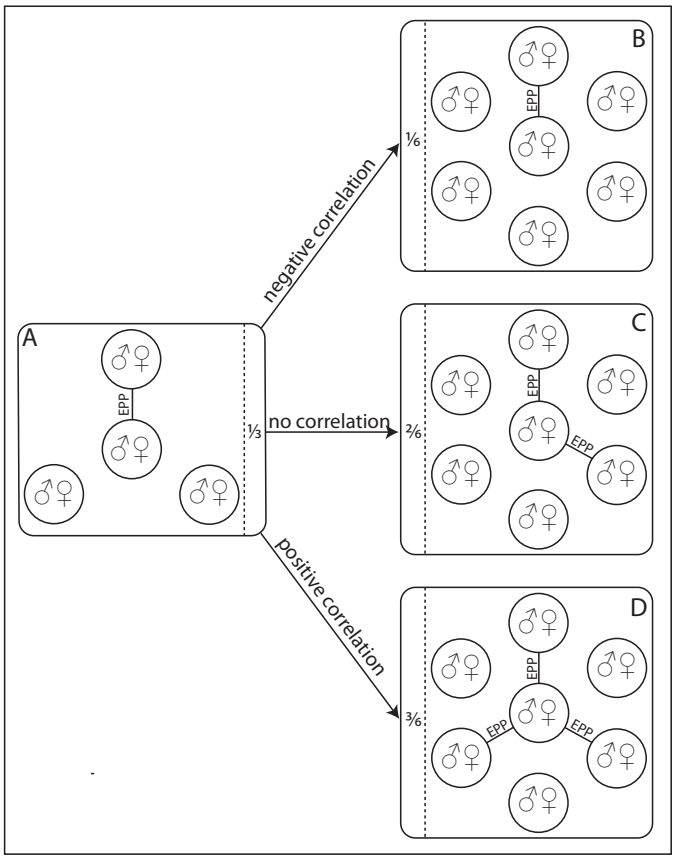

Figure 3. Schematic representation of scenarios that lead to different correlations between the number of neighbours and EPP. In the initial situation (A), a central individual (of either sex) is surrounded by 3 neighbours, with 1 EPP event occurring among the individuals. A third of the potential extra-pair matings is thus realized. When the number of neighbours increases (here: doubles), three scenarios are possible. (1) The proportion of realized EPP events decreases, leading to an overall negative effect of the neighbour number on EPP (B). (2) The proportion of realized EPP events remains constant, with no correlation between local density and EPP (C; the 'null' hypothesis). (3) The proportion of realized EPP events increases, leading to an overall positive effect of local density on EPP rate (D).

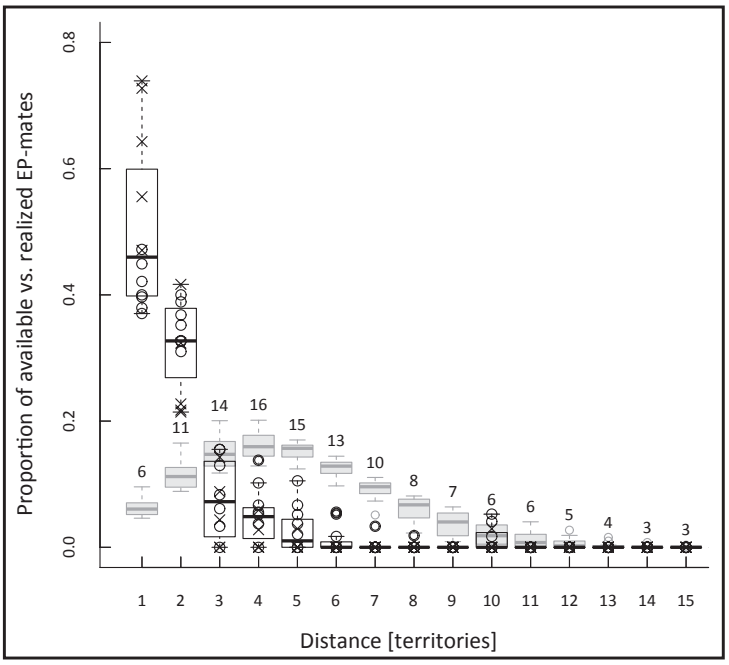

Figure 4. Distribution of EPP in relation to breeding distance in blue tits. The black boxplots show the realized distribution of EPP for the two populations (circles: Kolbeterberg, crosses: Westerholz) as the proportion of cases in each breeding distance class. Each breeding season is one data point. The grey boxplot represents the availability of EP mates in each breeding distance class in the population and thus reflects the expected distribution of EPP if it were a random process with respect to distance. Numbers above the grey boxplot indicate the average number of neighbours of an individual at the respective breeding distance.

Data selection

For both study sites, we included only cases where both the male and the female were known breeders (to be able to calculate breeding distance). This implies that we cannot make inferences about the non-breeding extra-pair sires, which may be floater males (true non-breeders), undetected failed breeders, or males that bred in natural cavities or outside the study area. However, the occurrence of unknown extra-pair sires was spatially independent in both study areas (significant spatial autocorrelation in only 1 of 12 seasons, low assortment coefficients in all years, Table S2). This means that broods with young from unknown sires were spatially independent, and not clumped within the population. Hence, it is unlikely that our results are biased due to co-variation between the distribution of unknown fathers and unmeasured environmental variables.

Statistical analyses

We used generalized linear mixed effect models (GLMMs) with a binomial error structure and a logit-link function and included the random effects 'male ID', 'female ID' and 'year'. The dependent variable 'occurrence of EPP' indicates for every male-female combination within a particular population and breeding season (except the social pair, by definition) whether they had EPO together (yes/no). We constructed models with one explanatory variable defined for the focal male-female combination (breeding distance). We included five explanatory variables for the males ('male perspective'): male age (population-wide effect), male body size (population-wide effect), male territory size (population-wide effect), male number of neighbours (population-wide effect), male asynchrony (relative synchrony of male-female pair at the respective breeding distance class; see above). Similarly, we included five explanatory variables for the females ('female perspective'): male age (age relative to that of the surrounding males), male body size (size relative to that of the surrounding males), female territory size (population-wide effect), female number of neighbours (population-wide effect), female asynchrony (relative synchrony of male-female pair at the respective breeding distance class; see above). We therefore had a set of eleven variables for analysis (Table 1). Please note that whether the male or the female neighbourhood is under focus will usually lead to different parameter values, because the male and the female that have EPP per definition do not breed on the same territory and therefore do not share their entire neighbourhood, and because EPP generally takes place on a local scale (see also Fig. 1, Fig. 2).

We first calculated correlation coefficients between all explanatory variables, because strong collinearities can bias the model output (Dormann *et al.* 2013 and references therein). Two pairs of parameters were strongly correlated ($r > 0.5$), namely 'male age' and 'male age relative to other males in the focal female's neighbourhood' as well as 'male body size' and 'male body size relative to other males in the focal female's neighbourhood' (Table S3). This indicates that in our specific setting we do not gain additional information by investigating male age and body size in a local context. This may reflect that in our population there is no spatial structure in male age and male body size, i.e. males are distributed randomly across the population with respect to tarsus length

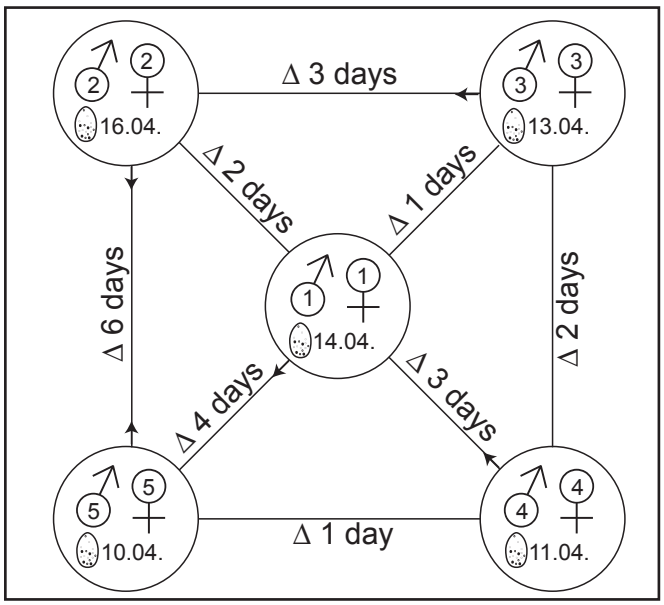

Figure 5. Visualization of potential differences between male and female neighbourhood effects of breeding asynchrony on EPP. Shown is a simplified example of a population of five social pairs with different laying dates. Considering only first-order neighbours, each pair 'perceives' the other pairs' asynchrony differently. If high asynchrony enhances the chances for EPP, the EPP events favoured from a female neighbourhood perspective ($♀_1 – ♂_5$, $♀_2 – ♂_5$, $♀_3 – ♂_2$, $♀_4 – ♂_1$, $♀_5 – ♂_2$) partly differ from the EPP events favoured from a male neighbourhood perspective ($♂_1 – ♀_5$, $♂_2 – ♀_5$, $♂_3 – ♀_2$, $♂_4 – ♀_1$, $♂_5 – ♀_2$).

and age (data not shown). All other correlation coefficients were well below the threshold of $r = 0.5 – 0.7$ (Dormann *et al.* 2013), indicating that here variation among potential EP mates was sufficient to investigate local male and female neighbourhood effects separately. We therefore included male age and male tarsus length on a population-wide scale as explanatory variables, while all other parameters were included as relative measures, i.e. relative to other individuals in the male and the female neighbourhood. We also assessed whether male age and body size relative to that of the respective female's social mate (difference or quotient between potential extra-pair male and social male) was a good predictor of EPP. However, this reduced our sample size by 10%, because not all social males had been caught. Because relative age and size were not better predictors of EPP than absolute values, we used the latter in the final model.

Of the original eleven potential explanatory variables we therefore excluded two ('male body size given female surroundings' and 'male age given female surroundings') due to strong collinearities, leaving a subset of nine variables in the final model. All explanatory variables, except 'distance class', were centred (see Table 1) and all variables were scaled ('standardized') by dividing each value by two times the population-wide standard deviation. The latter allows direct comparison of effect sizes among all variables, including two-level factors, such as male age (Gelman 2008). Thus, in our models, an increase by one reflects an increase by two standard deviations of the original data.

We aimed to investigate the effects of all variables while controlling for the spatial limitations of EPP. The relationship between breeding distance and the occurrence of EPP may take different shapes, but in our data is best described by two linear relationships with different slopes (for breeding distance 1-3, and 4-11, respectively; Fig. S1). We reduced our dataset such that it included only the first three breeding distance classes, because extra-pair sires rarely bred at larger distances (Fig. 4,

final sample size N = 316 broods with EPO, 398 male-female combinations with EPO) and controlled for breeding distance as a linear parameter (effect sizes were similar when including all distance classes, details not shown). We did not centre the variable breeding distance, because most EPP occurred at the first distance class. This implies that the model intercept is calculated at the first neighbouring distance class.

We compared the full models with all models where one parameter was eliminated using AICc values (Hurvich 1989). Since the full model performed as good as the other models (Table S4), we present only the results of the full model. Considering male-female combinations instead of individuals strongly reduces the proportion, but not the number of 'EPP events' in the dataset. However, this does not reduce the power of the tests (Table S5). Additionally, including every male and female numerous times into the same analysis in different male-female combinations did not inflate our Type I error rates when male and female identity were taken into account as random effects (function 'eppSimDat' in R-package 'expp', Valcu & Schlicht 2013).

All statistical analyses were performed using the free statistical software R 3.0.2 (R Core Team 2013). As supplementary material accompanying this study, we designed the R package 'expp' (Valcu & Schlicht 2013). This package provides a set of tools to perform the spatial data transformations required to obtain a data set as described in Fig. 1. It also provides several graphical methods facilitating data visualization. A data set and example scripts for clarifying data transformation and analysis are also contained in the expp package. For generalized linear mixed-effect models we used the R package 'lme4' (Bates & Maechler 2010). AICc tables were calculated using the R package 'AICcmodavg' (Mazerolle 2013).

Results

The further individuals bred apart, the less likely they had EPO with one another (Fig. 6, Table 2). Most extra-pair events occurred at the first neighbouring distance class ('direct neighbours') with a sharp decrease at higher neighbouring distance classes (Fig. 4).

Older males were more likely to sire EPO in both populations. Larger males tended to be more likely to have EPO overall, but not when the populations were modelled separately. The effect of male age and tarsus length decreased with increasing breeding distance, overall and in one of the study populations (Fig. 6, Table 2).

Having many neighbours reduced a male's chance of siring EPO with a specific female in his neighbourhood (overall, and in one study site, Table 2), indicating that an increase in the number of neighbours did not lead to a proportionate increase in EPP events. Overall, females with larger territories tended to be less likely to have EPO and males with larger territories tended to be more likely to have EPO (Fig. 6, Table 2); both effects were mainly driven by one study area. Breeding

Table 2. Effects of the explanatory variables on the occurrence of EPP in two blue tit populations. Shown are the estimates, standard errors, z- and P-values. Variables that differ for males and females are marked by symbols. Significant variables are in bold. Back-transformed estimates, which can be interpreted as probabilities, are presented in Fig. 6. See Methods for model details. Note that P-values were not corrected for multiple testing.

	Standard deviation of centred (not scaled) variable	Study areas combined			Kolbeterberg			Westerholz		
		Main effect								
		Estimate±SE	z	P	Estimate±SE	z	P	Estimate±SE	z	P
Intercept		**-3.27±0.12**	**-26.46**	**<0.001**	**-3.37±0.17**	**-20.37**	**<0.001**	**-3.10±0.18**	**-17.30**	**<0.001**
Breeding distance	0.76	**-2.24±0.16**	**-13.68**	**<0.001**	**-1.96±0.19**	**-10.15**	**<0.001**	**-2.67±0.31**	**-8.50**	**<0.001**
Male age (1st year)	0.59	**-1.46±0.16**	**-6.90**	**<0.001**	**-1.22±0.25**	**-4.95**	**<0.001**	**-1.87±0.42**	**-4.45**	**<0.001**
* distance		**0.70±0.30**	**2.33**	**0.02**	0.19±0.35	0.54	0.59	**1.32±0.60**	**2.20**	**0.03**
Male body size	0.52	**0.53±0.22**	**2.46**	**0.01**	0.45±0.27	1.64	0.10	0.55±0.34	1.61	0.11
* distance		**-0.62±0.28**	**-2.25**	**0.02**	**-0.84±0.32**	**-2.66**	**0.007**	0.12±0.58	0.20	0.84
Neighbour ♂	3.64	**-0.96±0.22**	**-4.46**	**<0.001**	**-1.28±0.26**	**-4.85**	**<0.001**	-0.41±0.39	-1.05	0.62
Number ♀	3.64	-0.10±0.19	-0.56	0.58	-0.15±0.22	-0.68	0.50	-0.18±0.36	-0.50	0.62
Territory size ♂	0.38	**0.30±0.16**	**1.93**	**0.05**	**0.47±0.20**	**2.32**	**0.02**	-0.22±0.26	-0.85	0.40
Territory size ♀	0.38	-0.27±0.15	-1.85	0.06	-0.34±0.18	-1.82	0.07	-0.17±0.24	-0.69	0.49
Asynchrony ♂	6.04	0.17±0.13	1.28	0.20	0.21±0.15	1.44	0.15	0.13±0.27	0.50	0.62
Asynchrony ♀	6.04	0.07±0.15	0.43	0.67	-0.07±0.18	-0.37	0.71	0.24±0.29	0.82	0.41

asynchrony did not influence EPP.

Effect sizes were comparable between the two study areas (Table 2, Fig. S2), except for breeding distance (interaction with study site: -0.75±0.38, z = -2.00, P = 0.05), male territory size (-0.69±0.33, z = -2.09, P = 0.04), and the male number of neighbours (0.89±0.48, z = 1.86, P = 0.06).

Of the variance explained by the random effects, less than 1% could be attributed to differences between seasons and study areas, whereas 74% and 26% was explained by male and female ID, respectively. This indicates that attributes of individual males were more important than attributes of individual females. Note that the effect of female identity cannot be separated from attributes of her social partner, unless his characteristics or identity are explicitly taken into account as explanatory variables or random factors.

Discussion

Including the spatial availability of mates into studies on EPP is important, especially when studying who mates with whom (e.g. Westneat & Mays 2005, Westneat & Stewart 2003). A consequent next step is therefore to change the focus from studying EPP of individuals towards studying the occurrence of EPP among all potential extra-pair partners. Here, we suggested a new modelling approach and illustrated it with a case study on blue tits. We investigated which of several variables - previously reported to explain patterns of EPP - influenced the probability that a given male-female combination within a breeding population has EPO together. We tested traits of individuals either at the population level, or relative to other individuals in the male and female neighbourhood. Using data from two independent populations, we discuss general and population-specific effects below.

General approach

Our approach, which focuses on pairs of birds instead of individuals, has several major advantages.

First, spatial effects (such as breeding distance) and effects that take place at a local scale (e.g. interactions among neighbours) can be modelled explicitly. For example, assume that one would like to test the hypothesis that larger males are more likely to sire EPO because they are more competitive or because they are preferred by females. Typically, the effect of male body size is modelled population-wide. However, if EPP is largely restricted to close neighbours, absolute size may be less important than size relative to the surrounding males. On the other hand, relative size is difficult to assess without defining a complex spatial framework as we describe it here. As long as the breeding location and size data of all individuals are known such local effects can explicitly be included in the model.

Second, variables that focus on the males (e.g. breeding asynchrony with the surrounding females; 'male EP gains') and variables that focus on the females (e.g. breeding asynchrony with the mates of the surrounding males; 'male EP losses') can be included at the same time, and their relative importance can be assessed in the same model. This is not possible with conventional methods, which usually focus only on one individual of the extra-pair (males or females). For example, we may want to investigate whether asynchronous breeding enhances EPP under two hypotheses. (a) The effect of asynchrony arises because males court only the most asynchronously breeding females due to a trade-off with mate guarding. In this case, the relevant variable is the asynchrony of the focal female relative to that of the other females surrounding the male. (b) The effect of asynchrony arises because females are more likely to have access to males whose female is incubating, e.g. because of female-female aggression. In this case, the relevant variable is the asynchrony with the mate of the focal male, relative to that of the mates of the surrounding males. Thus, different behavioural mechanisms can lead to different outcomes and thus effect sizes when tested from the male vs. the female perspective, as illustrated in Fig. 5. With the approach presented here, we can robustly test such hypotheses.

Third, the joint modelling of different types of traits allows controlling for various confounding factors. For instance, we can assess the effect of male age, territory size, or relatedness on EPP while controlling for breeding distance, which may be a confounding factor, for example if there is a strong spatial structure in male age, territory size, or quality (e.g. older males may breed clustered in high-quality areas of the habitat, Morton *et al.* 1990; related individuals may cluster together due to philopatry; Brouwer *et al.* 2011). Similarly, we can assess effects of local density on EPP while controlling for effects of local, pairwise asynchrony and vice versa. Because breeding distance is a parameter defined by a pair of individuals, controlling for breeding distance is generally not possible when focusing on traits of one sex only, unless the analysis is restricted to a comparison among individuals that had EPP at different distances (without taking into account non-realized potential extra-pair mates).

Fourth, the joint modelling of different types of traits allows a direct comparison of effect sizes. For example, both effects of male age and of breeding asynchrony on EPP appear in the literature, but their relative strength could not previously be investigated.

Finally, the approach may also be used to specifically test hypotheses that imply that a parameter's importance for EPP depends on the partner's breeding distance (see case study below).

Our approach also has practical and statistical limitations. (a) Obviously, only events can be included where the EP father can be assigned and his breeding location is known. This may restrict the usefulness in species and studies where many of the EPO are sired by males that do not breed inside the study area. (b) A general concern for this and other behavioural studies is that individuals – or in this case pairs of individuals – are treated as independent data points. This independency assumption is violated if an interaction among two individuals changes the future behaviour of these individuals, which is likely, at least to some degree. Generally, such 'social dependence' is neglected,

because it is not trivial to take it into account. Our method at least partly addresses this issue, because attributes and behaviour of potential interaction partners can explicitly be included in the model. (c) Calculating R^2-values, which are often used to assess the relative importance of variables, is not straightforward in models that include random effects or that follow a non-linear distribution (Nakagawa & Schielzeth 2013). We suggest instead to compare the importance of different variables by their relative effect size and significance, which should correspond to the 'traditional' R^2-value (Nakagawa S & Schielzeth H 2013 and references therein). (d) Special care should be taken to model only biologically meaningful effects, because the complexity of this type of model makes unexpected results (and potential type-I errors) even more difficult to interpret.

Case study

As expected, breeding distance had a large effect on the probability of having EPO. The probability that a potential pair had EPO decreased by 89% from direct neighbours to second-order neighbours (Table 2, Fig. 6), and few extra-pair sires bred further than two territories away (Fig. 4). Such a pattern is expected, because individuals have a higher chance to meet and interact with close-by individuals.

As shown in previous studies on blue tits and other species (e.g. Akçay & Roughgarden 2007 and references therein), male age was an important determinant of the observed EPP patterns. Older males were on average 4.1 times as likely to gain EPO than first year males, potentially reflecting male quality, female choice, or male investment into EPP (for further discussion see Akçay & Roughgarden 2007). Similarly, larger birds were more likely to have EPO (Table 2 and S1, Fig. S2), as shown previously in blue tits (Kempenaers, Verheyen & Dhondt 1997) and other birds (e.g. Akçay & Roughgarden 2007 and references therein). A previous study on the Kolbeterberg population, spanning the first four years, found that in a pairwise comparison, extra-pair males were older and larger than the social males they cuckolded, but only if they were close neighbours (Foerster *et al.* 2003). Here, we show explicitly that the effect of male age and body size decreases with increasing breeding distance (Table 2; no difference if pairwise comparison of male body size used, see methods). Our analysis thus suggests that the mechanisms driving EPP may differ depending on the breeding distance among individuals.

We expected that individuals with larger territories would be less likely to be involved in an extra-pair event, because a large territory may limit the encounter rate of an individual with potential EP mates (e.g. Westneat & Mays 2005, Westneat & Sherman 1997, Thusius *et al.* 2001). For females, effect sizes were indeed negative, but non-significant. However, in one population, males with larger territories (population-wide) tended to be more likely to have EPO (Table 2, Fig. 6). Such an effect could arise, for instance, if highly competitive males were able to secure both large territories and more EPO. Overall, our results suggest that individuals are not constrained in their extra-pair behaviour by having larger territories, at least for the territory sizes recorded in this study.

For males, we found a negative relationship between the number of neighbours and the probability

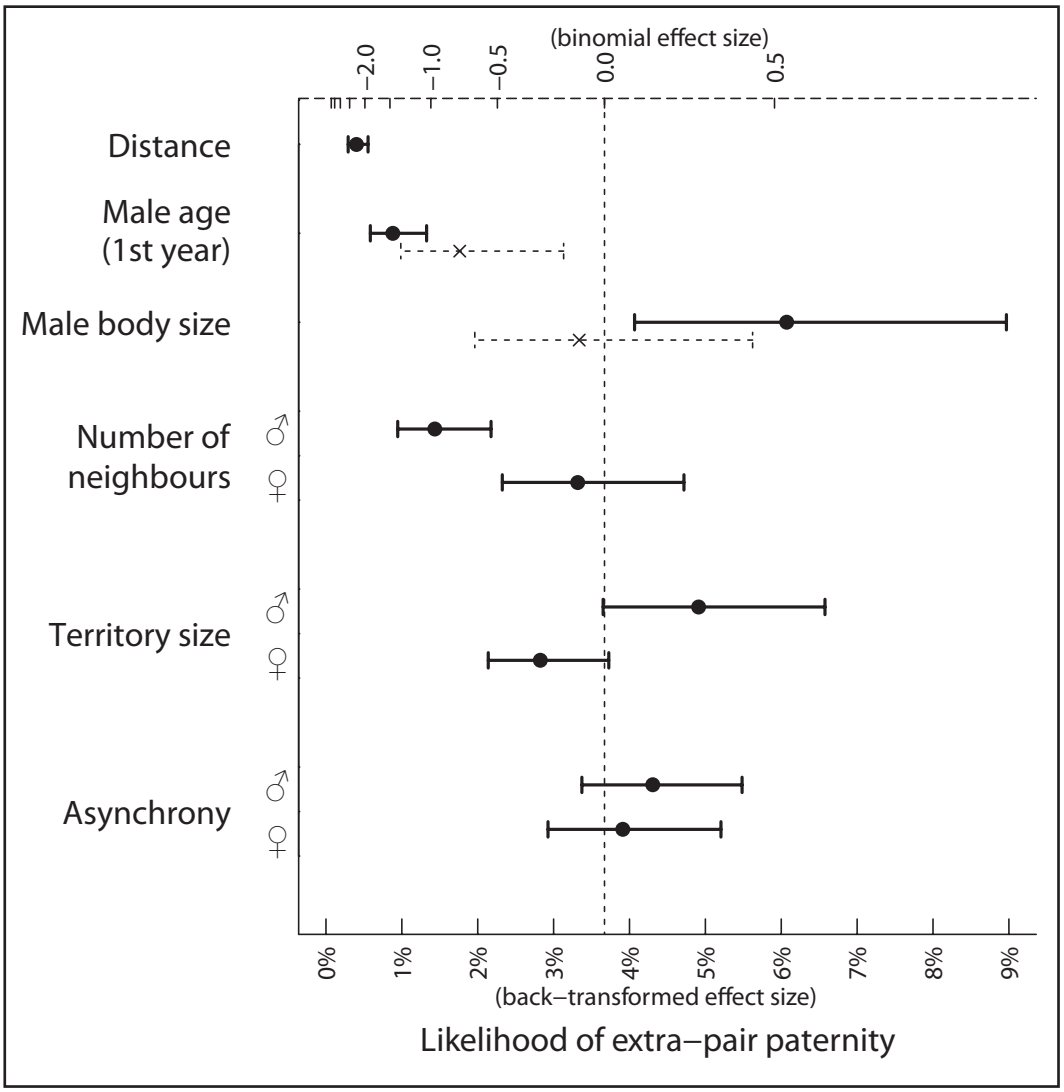

Figure 6. Effect sizes for all explanatory variables on the occurrence of EPP (yes/no) in two blue tit populations. Shown are the back-transformed parameter estimates and the confidence intervals, which can be interpreted as probabilities for an EPP event (bottom axis). The vertical dashed line indicates an effect size of zero and a baseline probability for a pair to have EPO. Interactions are visualized as binomial estimates for the main effect at a higher breeding distance (crosses and dashed lines). Note that the interactions can only be shown on the binomial and not on the backtransformed (probability) scale. A male-female combination of direct neighbours where the male is an adult (older than first year) and all other variables are intermediate therefore has an average probability of 3.4% to have EPO together. If the male was a yearling in the otherwise same situation, the probability decreases to 1.1%. The axis on the top refers to the binomial effect sizes to allow assessment of the non-back-transformed effect sizes. See Methods and Table 2 for model details. The male and the female neighbourhood are indicated by symbols on the y-axis.

of siring EPO (Table 2, Fig. 6). This means that an individual male with more neighbours sired EPO with fewer extra-pair mates than expected if the chance of extra-pair success were proportional to the number of available mates (Fig. 3), independently of potentially confounding effects such as male territory size, age, or body size. In previous studies that tested the relationship between number of neighbours and EPP, the slope is generally difficult to interpret (see Methods). In contrast, our results imply that the number of females with whom a male can sire EPO is limited by other factors than mate availability. This outcome can occur, for instance, if males are limited by the amount of courtship (or sperm) they can produce, and thus have a limited number of successful extra-pair copulations they can achieve, or if the number of successful encounters with potential EP mates does not increase proportionately with the number of neighbours. The effect of the female's number of neighbours had the same sign, but was non-significant. Because the effect sizes are similar, speculations about

different mechanisms acting on males and females seem unwarranted.

Breeding synchrony has been hypothesized to influence extra-pair mating patterns in two ways. First, a certain synchrony between breeding pairs may be necessary for extra-pair matings to occur, as it insures that sexually active potential mates are available (e.g. Chuang, Webster & Holmes 1999). Second, synchrony may reduce the opportunities for extra-pair matings in species that exhibit mate-guarding behaviour (such as the blue tit, Kempenaers, Verheyen & Dhondt 1995), because of a trade-off between investing in mate guarding and in extra-pair behaviour or courtship during the fertile period of the social female (e.g. Emlen & Oring 1977, Yezerinac & Weatherhead 1997, Chuang, Webster & Holmes 1999, Thusius *et al.* 2001, Canal, Jovani & Potti 2012, but see Kempenaers 1997). If this argument holds in blue tits, a larger asynchrony should facilitate the occurrence of EPO for any male-female combination. Since this reasoning is based on a trade-off for males, males are expected to be more strongly influenced by breeding asynchrony than females. However, our analysis shows that asynchrony was not linked to the likelihood of EPP in either study area, confirming an earlier study (Kempenaers 1997). This may indicate that there is no strong trade-off between mate-guarding and extra-pair behaviour in this population, as expected if EPP results from active female behaviour (Kempenaers *et al.* 1992, Kempenaers 1997).

Conclusions and outlook

In this study, we suggested a novel method that allows investigating which parameters influence the extra-pair mating patterns we observe, and that helps to formalize and test hypotheses about the potential mechanisms underlying such effects. The novelty of the approach is that it takes into account all potential male-female combinations that could have EPO, and investigates which of these potential "extra-pairs" are realized based on the respective male and female characteristics and the characteristics of their neighbourhoods. Moreover, this approach is useful when considering behaviours or traits that show spatial clustering. Results of a case study on blue tits, where we corrected for the breeding distance between individuals, indicated that from those variables we tested the most important factor driving EPP was male age (population-wide effect). Larger males were overall also more likely to sire EPO. Both effects decreased with breeding distance, indicating that different mechanisms may drive EPP between close neighbours and between individuals breeding further apart. Whereas breeding asynchrony did not influence EPP, one measure of breeding density (number of neighbours) did, but only for males. Our study therefore suggests that traits may have a different impact on EPP when considered relative to the focal male's or female's neighbourhoods.

We tested well-established hypotheses about EPP to illustrate a new approach to analyse interactions between individuals (here: whether a pair had EPO together). The method can be applied more widely to address a variety of questions, and is particularly useful (a) to test whether and how processes differ at different spatial scales, and (b) to test how traits of individuals, of the potentially interacting pairs and of their neighbourhoods influence the outcome of these interactions. Traits of the interacting pairs can include behavioural observations. For example, our approach allows

investigating whether pairs (excluding social mates) that interact more often in a different (e.g. foraging) context are more likely to have EPO with each other, given other known effects on the occurrence of EPP (e.g. male age, breeding distance).

Similar analyses can be conducted with other interactions than EPP as the response variable. For example, one could investigate parameters predicting which individuals form a social pair-bond, which individuals behave more (or less) aggressively towards each other, which individuals are more likely to forage together, etc. Furthermore, the same approach can be used to investigate underlying factors that can explain changes in the "value" of a pair over time, or to calculate the repeatability of this pair "value". For example, if multiple measurements on reproductive parameters (e.g. clutch size) or pair behaviour (e.g. relative amount of male care, proportion of paternity lost) are available, one can test whether the change in pair "value" can be explained by changes in aspects of the local neighbourhood (e.g. number of competitors). Thus, the approach we describe here can easily be extended to a wide range of questions in many behavioural systems.

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Data accessibility

All data are supplied with the R-package 'expp' (Valcu & Schlicht 2014; URL: http://CRAN.R-project.org/package=expp).

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Supporting Information

The following Supporting Information is available for this article online.

Table S1. Differences between the study areas.

Table S2. Spatial autocorrelation of the presence of unknown extra-pair fathers for all breeding seasons.

Table S3. Correlation coefficients of all variables.

Table S4. Comparison of the full model and reduced models using the AICc information criterion.

Table S5. Examples illustrating the relationship between probability of an event, sample size, and power in a binomial model.

Fig. S1. Models of the relationship between breeding distance and the occurrence of EPP events.

Fig. S2. Effects of all explanatory variables on the occurrence of extra-pair paternity in two blue tit populations separately.

Chapter 3

No relationship between female emergence time from the roosting place and extrapair paternity

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Original Article

No relationship between female emergence time from the roosting place and extrapair paternity

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In several bird species, the period around dawn seems important for extrapair behavior. For example, a study on great tits (*Parus major*) showed that females that emerged earlier from their roosting place during the peak of their fertile period were more likely to have extrapair young in their brood. We investigated the potential effect of female emergence times on extrapair behavior in the blue tit (*Cyanistes caeruleus*). First, we tested the relationship between natural female emergence times from the nest-box and the presence or frequency of extrapair offspring in the brood, using 4 years of data. Females progressively emerged earlier from the nest-box as egg laying approached, with the earliest emergence 2 days before the start of laying. However, we found no relationship between female emergence time and the occurrence of extrapair young in the brood. Secondly, in 2 breeding seasons, we experimentally advanced female emergence times by supplying the roosting females with additional light in the early morning. Although the experiment had inconsistent effects on the occurrence of extrapair young in the brood, we found no evidence that female emergence time during peak fertility is directly linked to extrapair paternity. Interestingly, females exposed to artificial light were more likely to return to breed in the next year.

Key words: **artificial lighting, emergence time, extrapair behavior, female behavior, roosting.**

INTRODUCTION

Most of the studied, socially monogamous bird species perform copulations outside the social pair-bond, leading to extrapair paternity (EPP) (Griffith et al. 2002). Ever since extrapair behavior was discovered in birds in the 1970s, it has received much attention because it opens up a new pathway via which sexual selection can occur through male–male competition and female choice. Yet, despite the many years of study, we are still far from a full understanding of the behavioral mechanisms underlying the occurrence of EPP.

Studies on a variety of songbirds have shown that extrapair behavior often occurs in the early morning hours, before or around dawn. Black-capped chickadees (*Poecile atricapillus*), for example, were observed to copulate outside the pair-bond during twilight (Smith 1988; Mennill et al. 2004). In superb fairy wrens (*Malurus cyaneus*), radio tracking revealed female extraterritorial forays before sunrise. These forays are likely to be linked to EPP (Double and Cockburn 2000). Captive female pied flycatchers (*Ficedula hypoleuca*) performed most extrapair solicitation displays in the early morning (Drevon and Slagsvold 2005). Finally, blue tit males that join the dawn chorus comparatively early were more likely to gain EPP, suggesting an influence of early morning behavior on EPP (Poesel et al. 2006).

The dawn period may be especially important because low light levels may 1) decrease foraging efficiency, thereby weakening potential trade-offs between foraging and extrapair behavior (as has been proposed for territory establishment and mate attraction, Verner 1965) and 2) decrease male mate-guarding efficiency as vision may be obscured, thereby increasing female extrapair mating opportunities (Double and Cockburn 2000), at least if males advertise their location by singing. Additionally, some male characteristics potentially relevant for extrapair mate choice, such as aspects of the dawn song (Poesel et al. 2006), or simply the location of the male (Double and Cockburn 2000) may only be assessed effectively around dawn. Furthermore, due to sperm depletion, females that acquire copulations from a male before other females may increase the amount of sperm they obtain (Double and Cockburn 2000).

If extrapair behavior is frequent in the period around dawn, then the availability of extrapair mates during this period and the timing of female emergence from the roosting place may majorly

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influence opportunities for extrapair copulations and, hence, the occurrence of EPP. Indeed, a study on great tits reported that females that had extrapair young (EPY) in their brood had emerged earlier from their nest-box during the period of peak fertility than females that did not have EPY (Halfwerk et al. 2011). Here, we report on a systematic investigation of the relationship between variation in female emergence times from the roost during the fertile period and the occurrence of EPP in the closely related blue tit. First, we test this relationship using correlative data spanning 4 breeding seasons. This approach does not allow to infer causality because natural female emergence times arise either through female decisions or through constraints on females. Therefore, we experimentally advanced female emergence times in 2 breeding seasons by supplying them with additional light in the early morning. This allowed us to specifically test the hypothesis that females that emerge earlier have more opportunities to engage in extrapair copulations and hence are more likely to end up with EPY in their brood.

METHODS

Study system and field procedures

We studied a population of blue tits (*Cyanistes caeruleus*) in a mixed deciduous/coniferous woodland ("Westerholz," 48°08′26″N, 10°53′29″E) near Landsberg am Lech, southern Germany. The study area is an unmanaged part of the forest ("Reiherschlag," ca. 40 ha), which is dominated by mature oak trees and contains 277 nest-boxes (since 2007) with 60–150 breeding attempts of blue tits each year.

Blue tits are small (ca. 10–12 g) hole-nesting passerines. Most blue tits are socially monogamous, but social polygyny is not uncommon (Cramp and Perrins 1993; Kempenaers 1994). In our study area, blue tits raise only 1 brood per year. Some pairs produce a replacement clutch after the first brood failed (such replacement broods are not included in the experiment). Blue tits can be found in our study area throughout the winter. Although dispersal occurs mostly before individuals breed for the first time (natal dispersal), short-distance adult (breeding) dispersal also occurs, mostly by females (Cramp and Perrins 1993; Valcu and Kempenaers 2008).

Each year, we monitored all nest-boxes to obtain data on the start of nest building, the start of laying, clutch size, hatch date, and brood success. We checked all nest-boxes weekly and those close to the start of laying or close to the expected hatching date daily.

For paternity analysis, we took blood samples (approximately 50 μL) from all adults feeding 8- to 10-day-old nestlings (captured on the nest) and from all nestlings when the oldest nestling was 14 days old. Since 2007, adult birds are also equipped with passive integrated transponder (PIT) tags at capture. Note that because our experiment took place before the adults were captured, only 44% of the males and 53% of the females had a PIT tag during our experiment (from the previous year). For a detailed description of the study site, general field procedures, and the paternity analysis, see Schlicht et al. (2012). Permits were obtained from the Bavarian government and the Bavarian regional office for forestry (LWF).

Female sleep and emergence times

In spring, male blue tits sleep less frequently in nest-boxes than females, and males generally do not sleep in the nest-box in which the female is building the nest, whereas females regularly do (Perrins 1979, p. 154; Steinmeyer et al. 2010; our unpublished data). We, therefore, assumed that the bird whose behavior we recorded at a respective nest-box was the focal female that bred in that box. We confirmed that this was the case in at least 87% of cases where the female was marked with a transponder (see below).

All nest-boxes were equipped with an automated recording system that registered movements through the entrance hole and their direction using 2 light barriers placed at the inside and the outside of the entrance hole. The system also contained a radio frequency identification reader (RFID) at the entrance hole, which automatically recorded all PIT-tagged birds at the nest-box entrance. Detailed information about the automated recording system can be found elsewhere (Schlicht et al. 2012). This system allowed us to determine whether a female slept inside a nest-box on a certain day (if the light barrier inside the nest-box was triggered first) and when she emerged from the nest-box in the morning. However, the interpretation of the light barrier system is not always straightforward because the light barriers may also be triggered by, for example, slugs or moths passing through the entrance hole, because the male may be visiting his roosting female (thereby triggering the outer light barriers, see also below), or technical issues may cause the system to malfunction. Thus, to assess the quality of the female emergence data obtained via this system, we additionally equipped a subset of 94 nest-boxes with sound recorders ("SongMeter SM2," Wildlife Acoustics Inc., see also Halfwerk et al. 2011) and microphones installed inside the nest-box. Emergence times of roosting individuals can easily be detected in these sound recordings both auditory and visually by inspecting the spectrogram (see Supplementary Figure S1 and Supplementary Audio S1). The sound recordings do not allow identification of the sleeping individual. To validate that the recorded birds were indeed the focal females, we investigated for PIT-tagged females whether their transponder was the first to be read at their nest-box in the morning. This was true in 87% of 4922 cases. In 12% of the remaining cases, a male identity was recorded first. This may indicate either that the male was visiting his female in the early morning, which often happens (and may also lead to an underestimation of female sleep nights; see our data below), or that indeed the male was sleeping in the nest-box. These 2 cases are difficult to disentangle because an individual approaching the nest-box may trigger the outer light barrier around the same time as the individual inside the nest-box triggers the inner light barrier, thereby confounding the directional information from the light barrier system. In the remaining 1% of the cases (52 nights, 10 females), another female was recorded first. Seven of these females apparently attempted to replace the local female and were successful in 5 cases. The remaining 3 females performed single short visits to another nest-box.

Emergence times obtained by the automated recording system and the sound recordings differed on average by 1.5 ± 0.4 min (mean ± standard error [SE]; range: −74.1 to 59.4; sample size: $N = 633$ mornings recorded at 74 boxes). Small differences can be explained by slightly differing clock settings of the respective systems, whereas larger errors are likely due to misinterpretation of the light barrier information. In 61 of 103 nights (59%) that were scored as "no sleeping female," the sound recordings showed that a bird was actually sleeping in the nest-box. This suggests that our system overall led to an underestimation of how often females slept inside their nest-box. In 30 of 602 nights (5%) that were scored as "sleeping female" nights, no bird was sleeping inside the nest-box according to the sound recordings. The latter error is critical for our main analyses as it leads to spurious emergence times and therefore adds noise to the data. Because the comparison of the

emergence time between the sound data and the data from the electronic nest-boxes (light barrier system) was overall consistent, we used only the latter for analyses.

The time period considered for analyses

All analyses on emergence times were performed for 3 different periods. 1) The data were restricted to the morning of the day prior to the female's first egg (day −1). We chose this day because it makes the data directly comparable to those reported by Halfwerk et al. (2011) on which our hypothesis is based. 2) Data were restricted to the morning 2 days prior to the female's first egg (day −2). We chose this day because females on average emerged earliest on that day (Figure 1 and Supplementary Figure S2), and therefore, effects of early emergence may be most pronounced. 3) We used the mean emergence time of a female between days −5 and −1 because we aimed to conservatively capture the females' fertile periods (following Kempenaers et al. 1995). We ended the period at day −1 because in blue tits, extrapair copulations mostly take place before egg laying starts (Magrath et al. 2009; Vedder et al. 2010; our unpublished data) and because females are emerging considerably later once they start laying (Figure 2), thus potentially decreasing any effects of early emergence on EPP.

The correlational study

For the correlational part of this study, we combined emergence data spanning over 4 consecutive years (2010–2013). In 2012 and 2013, half of the females were experimentally treated (see below) and were therefore excluded from the correlational analysis. For the most relevant time period (from days −5 to −1, see above), we obtained the following sample sizes regarding emergence times—2010: 61 females in 234 nights, 2011: 38 females in 94 nights, 2012: 55 females in 190 nights, and 2013: 45 females in 135 nights. Note that these are sample sizes for the emergence time data, whereas the sample sizes presented in Figure 2 include all data where we could assess whether a female was sleeping inside the nest-box or not.

The experimental setup

Equipment and general setup

We aimed to advance the emergence time of experimental females throughout their fertile period until egg laying started by supplying them with additional light in the early morning. To this end, a diffuse warm-white LED (Nichia NSPL515DS, Nichia Corporation, Tokushima, Japan; typical luminous intensity: 1.9 cd) was placed inside the nest-box directly underneath the lid and pointing toward the nest-box floor (distance to sleeping female: 8–18 cm, depending on nest height). The LED was connected via a cable, leaving the nest-box at the back side, to a timer (relay: EATON EASY512-DA-RC, Moeller GmbH, Bonn, Germany) and a 12-V battery, both placed in a plastic box on the ground at the tree trunk approximately 2 m below the nest-box. All devices were checked daily and adjusted to the change in the time of sunrise, and batteries were exchanged after 2 weeks (if necessary). Nest-boxes of control females were also equipped with a LED and a (shortened) cable, but no electronics (timer and battery) were attached to the cable. Control boxes were also exposed to daily visits of the same length as experimental boxes to ensure equal treatment of the 2 groups. All experimental procedures were kept identical between years.

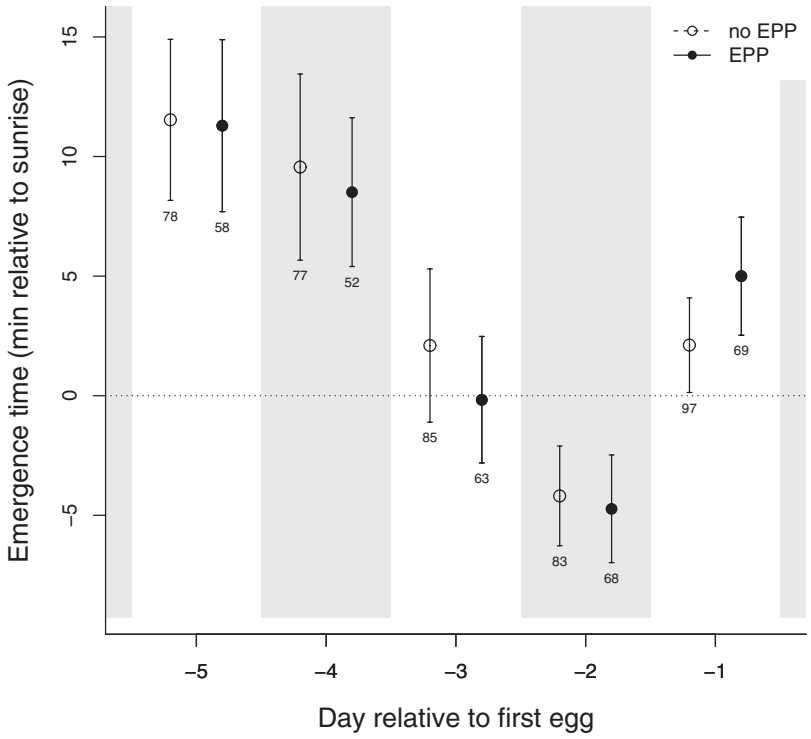

Figure 1
Difference in emergence times of females with and without EPY in relation to the start of egg laying (day 0). Shown are means and SEs. Sample sizes are given below the bars.

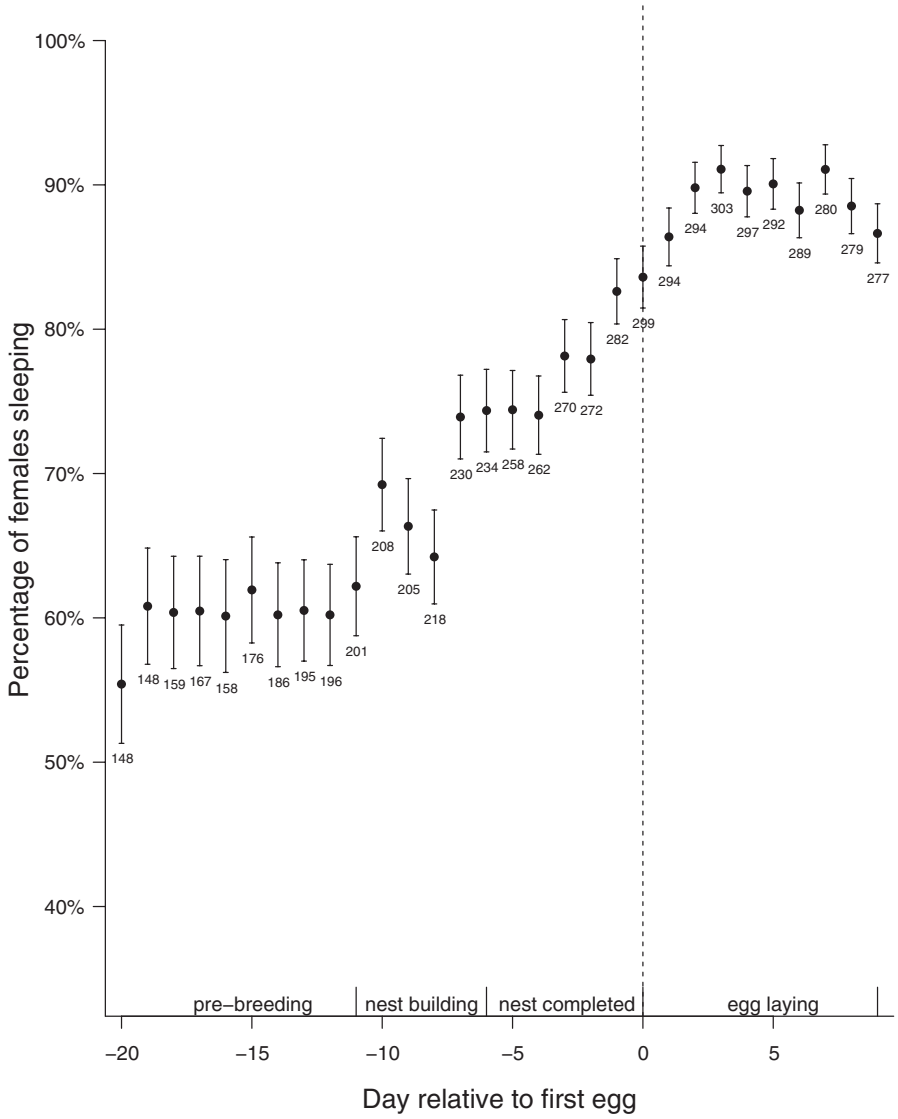

Figure 2
Percentage of females that slept inside the nest-box in relation to the start of egg laying (day 0). Shown are means and standard errors for individual days based on the pooled data from 2010 to 2013. Sample sizes below the vertical bars refer to the total number of mornings recorded. The indication of breeding stage above the *x* axis is based on average dates for all nesting attempts between 2010 and 2013 ("prebreeding": period before the start of nest building; "nest building": period between the start of nest building (at least bottom almost covered) and nest completion (nest cup with soft lining); "egg laying": females usually lay 1 egg per morning with an average clutch size of 10 eggs). Note that these are likely underestimations of the true proportion of females sleeping inside the nest-box (see Methods).

Seasonal timing

As stated above, the most relevant time period for extrapair copulations in blue tits is the time shortly before egg laying. Because we cannot predict when an individual female will lay her first egg, we installed all equipments on the day after we found a nesting attempt in a specific nest-box (nest-box floor completely or almost completely covered with moss). We turned off all experimental devices on the day the second egg was discovered because at that time extrapair behavior is expected to strongly decrease (Magrath et al. 2009) and we wanted to avoid further female disturbance during egg laying. Nest-boxes were thus lighted for a total of 2–31 mornings (mean ± SE—2012: 13.5 ± 0.7 mornings, $N = 113$ boxes; 2013: 9.8 ± 0.7 mornings, $N = 79$ boxes).

Daily timing

Preliminary tests in 2011 indicated that females did not leave the nest-box immediately after the light was turned on but that they did advance their emergence time compared with females in non-lighted boxes. Because males on average start to sing around half an hour before sunrise (Poesel et al. 2006; our unpublished data), and because natural emergence times for individual females varied between 40 min before sunrise and 97 min after sunrise (our data, see also Supplementary Figure S2), we turned on the light in the box 1 h prior to sunrise. We turned the light off 10 min after sunrise, when twilight was over and most females (>70%) had left their nest-box under natural conditions (our data, Supplementary Figure S2).

Assignment of treatment

To produce a uniform spatial distribution of treatments (as much as possible), we grouped new nests in clusters of close-by (< 160 m apart) nest-boxes. The first nest-box of each cluster was randomly assigned as "control" or "experimental" (by throwing a coin), unless treatment was determined by the treatment in the previous year (see below). Within each cluster, "control" and "experimental" treatments were then assigned alternately.

In 2013, we further aimed to treat females that had been part of the experiment in 2012 with the opposite treatment than the year before. This was only partly possible because more "experimental" than "control" females from 2012 returned to breed (see below). We, therefore, aimed to assign "control" and "experimental" treatments such that sample sizes for control and experimental groups were similar for new females and for females previously exposed to the experiment. In total, we ended up with 100 experimental broods (2012: 61; 2013: 39) and 103 control broods (2012: 58; 2013: 45). Of the 34 females that were exposed to the experiment in both years, 27 were treated as "experimental" in 2012, of which 19 switched to "control" treatment in 2013; of the 7 "controls" from 2012, 6 were assigned "experimental" treatment in 2013.

Impact of experimental disturbance

In 2013, we attached infrared cameras to the roof of the nest-box to record the behavior of experimental females inside the nest-box when the lights went on (for details on the camera system, see Steinmeyer et al. 2010). We recorded 3 experimental females over a total of 13 nights. For comparison, we also recorded 4 control females during the same time period. In 11 of the 13 nights of experimental recordings, the female was still in the sleeping position (head under the wing) when the light went on and did not immediately react to the additional light (Supplementary Video S1). On awakening, all females (experimental and control) engaged in behaviors such as preening, working on the nest, and stretching (Supplementary Video S2). In 3 experimental cases (2 females), the female went back to sleep (head under the wing) while the light was on. None of the females investigated the light source by looking at it for a prolonged time period or by flying up to inspect it. In 2 of 13 sampled nights, the female was awake when the light went on; in both cases, the female looked up briefly and continued the previous behavior (Supplementary Video S3).

To assess whether the experimental lighting disturbed the females, which might affect the interpretation of this experiment, we compared desertion rates among control and experimental females. For both years combined, 88% of experimental females (2012: 95%; 2013: 80%), and 90% of control females (2012: 93%; 2013: 87%) continued breeding. Experimental lighting, therefore, does not seem to induce abandonment of breeding attempts.

Statistical analyses

Emergence times were always centered within "day relative to female first egg" (e.g., by subtracting the mean female emergence on day −5 from a specific female's emergence time on day −5) to control for differences in emergence times due to the breeding stage. We used binomial generalized linear mixed-effect models with "year" and "female identity" as random factors, where necessary. For the models investigating effects of the emergence times or the experimental treatment on EPP, we used "EPP" (yes/no, or proportion of EPY in the brood) as the response variable. To investigate the effect of the experimental treatment on the time the female left the nest-box, we used "emergence time" as the response variable (Table 2). The model included the explanatory variable "treatment" as a factor with 4 levels: "experimental females during treatment" were compared with "control females during treatment," "experimental females before/after treatment," and "control females before/after treatment" (see below). We present model details together with the respective results. We used the free software R 2.15.1 (x64, R Core Team 2012) for statistical analyses with the package "lme4" (Bates et al. 2012) for mixed-effect modeling. We calculated repeatabilities of female emergence times both within and across years excluding experimental females and using the software version R 3.0.2 (x64, R Core Team 2013) and the package "rptR" (Schielzeth and Nakagawa 2013), which calculated repeatability values and confidence intervals based on a restricted maximum likelihood method (REML). Estimates and *P* values (based on permutations) were calculated using 1000 bootstrapping and permutation runs.

To assess within- and between-female influences of the experimental lighting on emergence times, we performed 2 comparisons. 1) We compared emergence times of experimental females on days when they were not lighted (e.g., because the nesting attempt had not yet been discovered; hereafter termed as "before or after lighting") with emergence times when they were lighted (hereafter termed as "during lighting"). 2) We compared emergence times of experimental females during lighting with those of control females during the same period.

RESULTS

Nest-box roosting and EPP

From 3 weeks before laying started until the first egg was laid, the percentage of females that slept inside the nest-box increased from about 60% to more than 80% (Figure 2). Surprisingly, only about 90% of females slept inside the nest-box during egg laying. Although this is probably an underestimate of the true percentage of females sleeping (see Methods), the observation of a female arriving at the nest-box before sunrise during egg laying suggests that females indeed do not always sleep inside the nest-box even during their laying period (personal observation).

The presence of EPY in a brood was unrelated to the number of nights the respective female slept inside the nest-box prior to laying (days −5 to −1; females with EPY: 3.25 ± 0.26 nights with sleep, females without EPY: 3.43 ± 0.17 nights with sleep, mean ± SE; *t*-test: $t_{272.29} = -0.98$, $P = 0.33$).

Repeatability of female emergence times

The within-year repeatabilities of emergence times were significant and ranged between $r = 0.16$ (2011) and $r = 0.37$ (2013; Figure 3); the between-year repeatability was nonsignificant ($r = 0.15$; Figure 3).

Female emergence times and EPP

Until about 5 days before egg laying started, females naturally emerged on average 26.7 ± 0.6 min after sunrise (mean ± SE, $N = 2365$). This changed dramatically in the last days before egg laying, with the earliest emergence on day −2 (mean ± SE: −4.4 ± 1.5 min; Figure 1 and Supplementary Figure S2).

Independent of the period considered, the probability that a female had EPY was not related to her emergence time (Table 1). This was true when data from all 4 years were combined (Figure 1, Supplementary Figure S2, and Table 1), when the nonexperimental years 2010 and 2011 were pooled (Table 1) or when the years

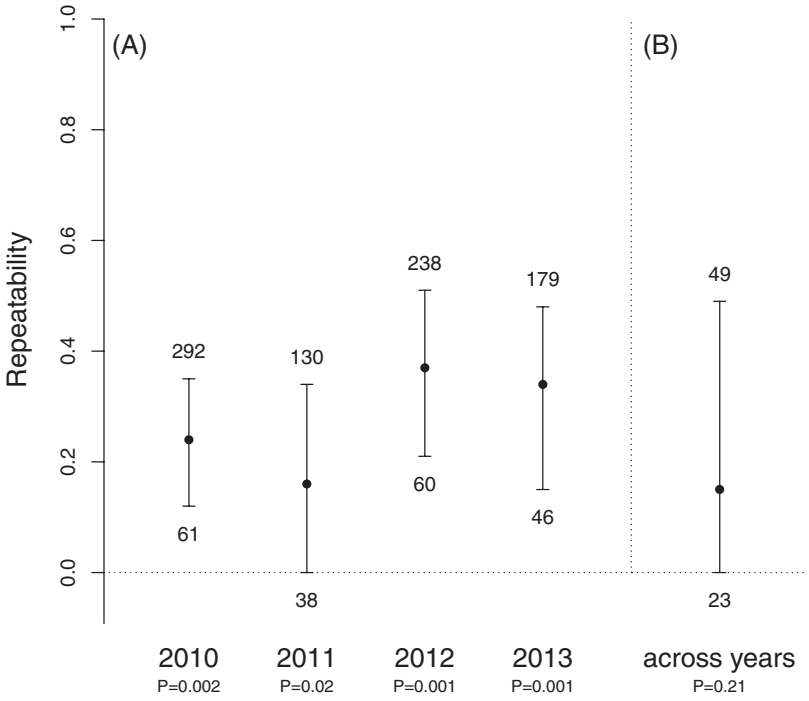

Figure 3
Repeatabilities of female emergence times (A) within years and (B) among years. Shown are estimates and confidence intervals. Sample sizes shown above and below the bars are number of data points and number of individuals, respectively. Repeatabilities were calculated for the time interval between days −5 and −1. Mean emergence times for each female relative to the population-wide expected emergence time on the respective day in relation to laying (see Methods) were used for calculating repeatability across years.

Table 1
Relationship between natural emergence times of females and EPP

	Period	Sample size	Intercept	Estimate ± SE	z	P
All years combined	Day −1	150	−0.40	0.57 ± 0.53	1.07	0.28
	Day −2	134	−0.24	−0.12 ± 0.59	−0.19	0.85
	Days −5 to −1	199	−0.23	−0.08 ± 0.41	−0.19	0.85
2010 and 2011 combined	Day −1	78	−0.74	1.29 ± 0.90	1.43	0.15
	Day −2	68	−0.50	−0.13 ± 1.10	−0.12	0.91
	Days −5 to −1	99	−0.42	0.52 ± 0.67	0.77	0.44

Shown are estimates from a binomial generalized linear mixed-effects model using "EPP" (yes/no) as the response variable and "emergence time" (relative to the other females on the specific day in relation to egg laying) as the explanatory variable. "Year" and "female identity" were included as random factors. The data were restricted to the day prior to the start of laying (day −1), 2 days prior to laying (day −2), and to the mean emergence on days −5 to −1, respectively. Estimates were calculated for all years pooled (top) and for the nonexperimental years (2010, 2011) pooled (bottom). Sample sizes differ because not all females were sleeping inside the nest-box every night.

were analyzed separately (details not shown; effect sizes in different directions, all $P > 0.07$). The proportion of extrapair offspring in a female's brood was also unrelated to her emergence time (independent of the period considered, no effects in individual years or in all years combined; all $P > 0.07$, details not shown). Furthermore, variation in emergence time did not explain any variation in the proportion of EPY in the brood when only considering females that had at least 1 EPY (all $P > 0.15$, years analyzed separately and combined). Moreover, emergence times of females that had EPY were unrelated to the distance (in meters) to the respective extrapair mate (Supplementary Table S1).

The experimental light treatment significantly advanced emergence times by on average 20 min, both between and within females (Figure 4, Supplementary Figure S3, and Table 2). Both between- and within-female effects were similar in both years (interactions with "year" nonsignificant with $P > 0.20$).

Unexpectedly, the experimental treatment influenced the likelihood that a female had EPY in opposite directions in the 2 years (Table 3; estimate ± SE of interaction term with year: −1.97 ± 0.59, $z = 3.32$, $P < 0.01$). In 2012, illuminated females were significantly less likely to have EPY, in contrast to what would have been predicted from the hypothesis that early emergence facilitates extrapair behavior. In 2013, illuminated females were significantly more likely to have EPY (Figure 5). The same but weaker effects were found when investigating how the experiment influenced the proportion of EPY (all $P < 0.13$). The effects disappeared when only females that had at least 1 EPY were considered (all $P > 0.10$). All effects increased (rather than decreased) with associated lower P

Table 2
Effect of the experimental light treatment on female emergence times

	Sample size	Estimate ± SE	t	P
Intercept (experimental during treatment)	694	−0.31 ± 0.04	−6.96	<0.001
Control "during treatment"	732	0.32 ± 0.02	16.04	≪0.001
Experimental before/after treatment	3235	0.35 ± 0.03	11.67	≪0.001
Control "before/after treatment"	3313	0.33 ± 0.03	13.13	≪0.001

Shown are estimates from a linear mixed-effect model using "emergence time" (relative to sunrise) as the response variable and with treatment as the explanatory variable. Shown are the contrasts between the group "experimental females during treatment" and the 3 control groups. "Year" and "female identity" were included as random effects. We conservatively used the number of females (166) as the degrees of freedom to calculate P values.

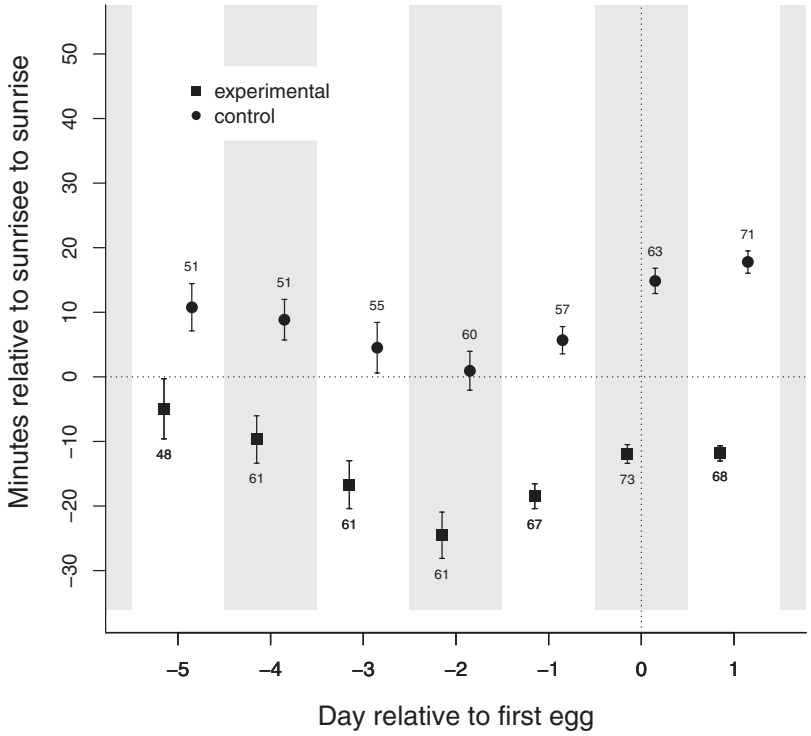

Figure 4
Influence of the experimental light treatment on emergence times. Shown are means and SEs. Sample sizes are given below (experimental) or above (control) the vertical bars.

values when including only females that were part of the experiment in just 1 of the 2 years (details not shown). Finally, variation in emergence time of the light-treated females only, that is, their individual response to the treatment, between days −5 and −1, was unrelated to the likelihood that they had EPY (all $P > 0.10$).

Further effects of the light treatment

Interestingly, 44% of the 2012 experimental females bred again in 2013 versus 14% of the 2012 control females (Fisher's Exact test: odds ratio = 0.33, $P = 0.01$) suggesting that experimental lighting may have increased return rates of females.

Females may perceive the additional light as an elongated day, and this may lead to a shift in the females' seasonal timing, thereby advancing laying dates. However, we found no strong influence of the experimental light treatment on the start of egg laying (t-test; mean difference: experimental broods 0.1 days earlier, $t_{201.26} = -1.71$, $P = 0.09$) and no influence on clutch size (t-test; mean difference: 0.2 eggs more, $t_{195.45} = -0.72$, $P = 0.47$). Interestingly, the light treatment seemed to have influenced the circadian rhythmicity of the females because experimental females emerged earlier than control females for several days after the exposure to illumination had ceased, at least until the end of egg laying (on average 4.2 min, Supplementary Figure S3; linear mixed-effect model, $t_{146} = -5.29$, $P < 0.001$, degrees of freedom conservatively defined as the number of females).

DISCUSSION

Effects of variation in natural emergence times

The aim of this study was to investigate whether females that emerged earlier in the morning from their roosting place during their fertile period were more likely to have EPY. A previous study on great tits (*Parus major*) suggested such an effect when considering the day prior to the female's laying start (Halfwerk et al. 2011). However, our extensive correlative data set did not confirm such an effect, neither when emergence time on the same day (day −1) was

taken, nor when the day of natural earliest emergence was considered (day −2), nor when emergence within a wider period was considered (days −5 to −1; Figure 1, Supplementary Figure S2, and Table 1). Females may need to emerge early only when their preferred extrapair mate is breeding further away. However, this is not supported by our results because the distance to the extrapair mate was unrelated to the emergence times of the females.

In 2012 and 2013, half of the females were illuminated. Although they were not included into this analysis, the experiment may nonetheless have influenced hormone levels or stress of all females, either through the disturbance caused by the experiment or via interactions of control females with experimental females. However, no effect could be found when restricting the data set to the 2 nonexperimental years, indicating that there is no general effect of natural emergence times on EPP. Interestingly, however, females generally emerged relatively early right before egg laying (Figure 1 and Supplementary Figure S2). Earlier emergence times may arise, for example, from energetic demands and needs for additional food intake in the days before egg laying starts. On the other hand, this dip in emergence time may point toward a different mechanism via which early morning behavior may influence EPP: females may generally emerge early during the period shortly before laying in order to pursue extrapair copulations. If a large majority of females performs this behavior, and if traits or behaviors other than emergence times (e.g., female ability to locate an EPP male) or postcopulatory processes (e.g., sperm competition) determine which females acquire extrapair fertilizations, variation in emergence times among females will not predict EPP despite a clear link between early morning behavior and EPP. Hence, we cannot conclude from our data that early emergence in the days before laying is not an important behavioral mechanism for EPP. We can conclude, however, that the natural between-female variation in emergence times does not explain variation in EPP.

We found significant repeatabilities of female emergence times within years, but not between years (Figure 2). A previous study on the same population of blue tits in earlier years found a strong repeatability of emergence times (both sexes combined: $r = 0.41$; Steinmeyer et al. 2010). Our within-year estimates were overall lower (0.2–0.4), potentially because—in contrast to the previous study—we only included females in our analysis, and females appear to have lower repeatabilities of emergence times than males (Steinmeyer et al. 2010). We had only relatively few nonexperimental individuals breeding in more than 1 season because we aimed to expose individuals to opposite treatments in both study years. Our estimate of between-year repeatability may thus be less accurate than estimates reported in the previous study (Steinmeyer et al. 2010). Both studies clearly suggest that emergence times in blue tits are an individual-specific trait.

If female extrapair behavior is constrained by the guarding male, then a highly variable emergence time of individual females could

Table 3
Effect of the experimental light treatment on EPP

	Estimate ± SE	*z*	*P*
Intercept (control 2012)	0.33 ± 0.27	1.21	0.23
Versus experimental 2012	−1.02 ± 0.39	−2.65	0.008
Versus control 2013	−1.02 ± 0.42	−2.45	0.01
Interaction: experimental 2013	1.97 ± 0.60	3.32	0.001

Shown are estimates from a binomial generalized linear mixed-effects model using "EPP" (yes/no) as the response variable and "treatment" in interaction with "year" as explanatory variables. "Year" and "female identity" were included as random effects. The model is based on 203 broods.

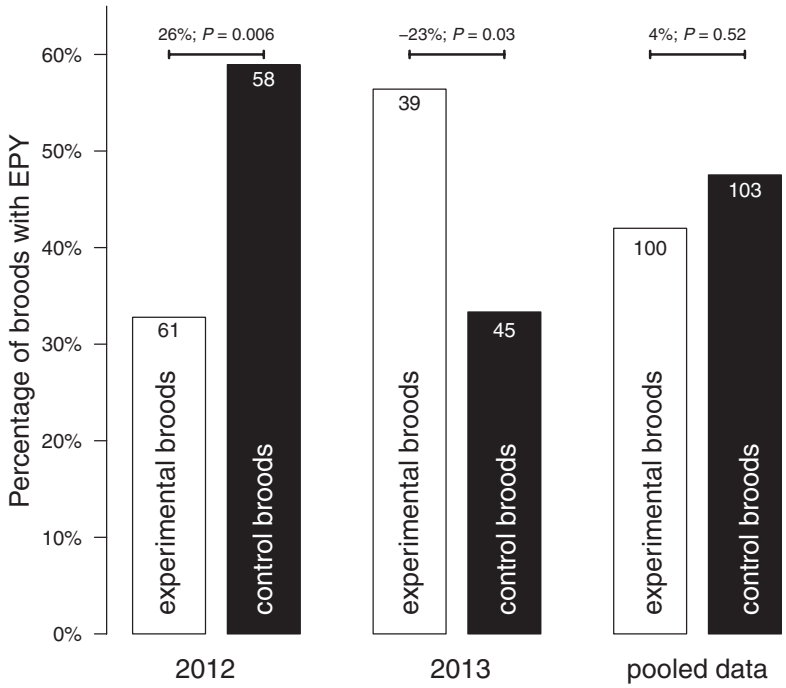

Figure 5
Influence of the experimental light treatment on EPP. Shown are the percentages of broods with EPY for experimental and control groups for both years separate and pooled. Differences between treatments and significance levels from the model are given above the bars. Sample sizes are shown inside the bars.

decrease mate-guarding efficiency, thereby increasing the likelihood for the female to have EPP. However, we found no effect of variance in female emergence time (female "predictability") during the 5 days prior to laying on EPP (binomial generalized linear model; all $P > 0.27$).

Effects of the light treatment

Although the light treatment advanced the emergence times of experimental females to the same extent in both years (Figure 4, Supplementary Figure S3, and Table 2), the effect on the likelihood of a female to have EPY was opposite between the years (Table 3). In 2012, illuminated females emerged earlier but—in contrast to the predicted effect—were less likely to have EPY. In 2013, illuminated females also emerged earlier and were more likely to have EPY (Figures 4 and 5). These results are difficult to explain. We may have picked by chance specific subsets of females with different intrinsic propensities to engage in extrapair copulations in the 2 study years, thereby producing inconsistent results, because we aimed to give females opposite treatments if they were part of the study twice. In this case, however, any effects should be weaker when restricting the data set to those females that were part of the experiment only once. This was not the case. Furthermore, extrapair behavior of one bird in the population is not necessarily independent of the extrapair behavior of other birds in the population. By changing the likelihood for an experimental female to have EPP, we may thus have changed the surrounding control females' likelihood to obtain EPP, thereby inflating type I error rates.

Alternatively, the experiment may have causally affected EPP in opposite directions in the 2 years, via an effect on unmeasured parameters (e.g., hormonal state), not via female emergence time. The 2 years differed in at least 2 aspects. 2012 was the year with the highest breeding density ever measured (almost twice as many breeding attempts as in 2013), resulting in high competition for nest-boxes. High breeding density may increase extrapair mating opportunities (Petrie and Kempenaers 1998) and may thereby influence the behavioral mechanisms via which EPP occurs, potentially leading to different results for high- versus low-density years. In 2013, on the other hand, females started to lay eggs on average 8 days later than in all other years (including 2012). Therefore, in 2013, sunrise was on average 15 min earlier during the prelaying period, causing an alteration in day length and light availability in the morning, and potentially influencing costs and benefits of EPP behavior in the early morning. The differences between years in the relationship between illumination and EPP may, therefore, also have been caused by the delay of breeding activities in 2013.

The link between female emergence times and extrapair behavior

A link between variation in emergence time among females and EPP may have several underlying causes. Firstly, female emergence time may directly influence EPP, for example, because fertile females that spend more time in the mating pool in the morning may have a higher chance to meet any or an adequate extrapair mate. In this case, we would expect that early emerging females (naturally and through experimental manipulation) should be more likely to have EPY. This was not the case, neither for control nor for experimental females (Figures 1 and 5, Supplementary Figures S2 and S3, and Tables 1 and 3).

Secondly, females with a high intrinsic propensity to engage in extrapair behavior may emerge earlier in the morning in order to pursue extrapair copulations (female decision). In this case, early emergence times should have been linked to a higher probability of EPP in the correlational data. This effect was not found (Figure 1, Supplementary Figure S2, and Table 1). To experimentally test this second hypothesis, female propensity to engage in extrapair copulations would need to be manipulated. This is not only difficult and beyond the scope of this study but is also less important because the extensive correlational data set fails to find any such link.

A third alternative could be that females would advance their emergence times in order to pursue extrapair copulations, but that ecological or physiological constraints, or their intrinsic daily rhythms, prevent them from doing so. Because illuminated females clearly emerged much earlier (Figure 4, Supplementary Figure S3, and Table 2), the experiment would have lifted such constraints, and hence, the experimental females should be more likely to have EPY. Although such an effect was found in 1 year, it was clearly absent (in fact opposite) in the other, ruling this hypothesis out as a general explanation (Figure 5, Supplementary Figure S3, and Table 3).

As a last scenario, females may leave their roosting place at a specific time in order to meet with a specific extrapair mate. In this case, lighted females, which emerged earlier due to the experimental treatment, should generally be more desynchronized with their extrapair mate, resulting in a negative effect of the light treatment on EPP. Again, although in one of the years such an effect was found, it was not present in the other, and we can therefore exclude this as a general explanation (Figure 5 and Table 3).

In contrast to our results, a previous study on the closely related great tit (*P. major*, Halfwerk et al. 2011) found that females that had EPY emerged on average 17.5 ± 4.8 min before sunrise, whereas females without EPY emerged 0.04 ± 5.71 min after sunrise, a statistically strongly significant difference. Although we cannot ultimately say why our results differ, there are 3 obvious differences between the 2 studies. 1) The sample size and the intent of the study. The study of Halfwerk et al. (2011) was designed to experimentally test effects of noise on the song of male great tits and on female emergence times. Emergence times were observed in a smaller sample of 22 females, making it more likely that the observed effect is a type I statistical error. Moreover, some females were exposed to experimentally elevated noise levels, which may have affected both emergence time and EPP. 2) The study species. Blue tits and great tits may differ in their behavior regarding EPP. Studies on different species would be needed to determine general patterns regarding a relationship between variation in female emergence times and EPP. 3) The study site and year. Although we did not find any clear relationship between natural emergence times and EPP, our experimental results suggest that under specific (unknown) circumstances, either the additional light itself or the resulting advances in female emergence times may increase the likelihood of obtaining EPY. Although both studies took place in mixed-deciduous habitats with nest-boxes provided, they were conducted in different geographical regions (The Netherlands vs. southern Germany) and partly in different years (2009–2010 vs. 2010–2013). Thus, it is likely that many environmental parameters differed between the 2 studies, which could potentially have led to different findings.

Further impacts of the experimental light treatment

Interestingly, the exposure to artificial lighting advanced female emergence times even after the experimental lighting was discontinued and changed their circadian rhythmicity at least for another week. Although we could not find fitness consequences of this shift in circadian

rhythmicity in terms of laying dates and clutch size, our approach of lighting roosting females could be used to investigate specific effects of circadian rhythmicity on the behavior or life history. An altered circadian rhythmicity may—either via advanced emergence times or via another pathway—also have induced the higher return rates of illuminated females. Increased local return rates imply either reduced breeding dispersal or increased survival of illuminated females.

CONCLUSIONS

In conclusion, we find no consistent effect of female emergence times from their roost on EPP either in the correlational or in the experimental data. This is in contrast with previous findings in the great tit and suggests that there is no direct causal link between variation among females in emergence time during the fertile period and the likelihood of having EPY, at least in blue tits.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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CHAPTER 4

Male extra-territorial behavior predicts extra-pair paternity in blue tits, *Cyanistes caeruleus*

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Lay summary

Blue tits typically breed as monogamous pairs. However, some individuals engage in extra-pair copulations, and as a result males can sire offspring with females that are paired to another male. We recorded all visits of individuals at nestboxes of other pairs. We found that extra-territorial visits are common, in particular close to egg laying. If a male visited a nestbox of another female, he had a higher probability to sire extra-pair offspring with her.

Abstract

In territorial species, at least one of the two individuals involved in an extra-pair mating has to leave its territory in order to interact and copulate with the extra-pair partner. However, data on extra-territorial forays are difficult and time-consuming to acquire, and therefore extensive and continuous datasets on foraying behaviour are rare. Here, we use data from an automated recording system to investigate forays of individual blue tits (*Cyanistes caeruleus*) to other pair's breeding boxes during early spring, when extra-pair matings occur. The study has three main aims. (1) To describe daily and seasonal variation in the frequency of foraying behaviour. (2) To investigate which individuals perform such forays. (3) To investigate whether male and female visits to other pair's nestboxes predict the occurrence of extra-pair paternity. We find that males visited foreign nestboxes more frequently than females, and that the majority of visits occurred in the morning hours, but not around dawn. Males were more likely to visit a foreign nestbox when their social female was in the later laying stage. Male body size, but not male age predicted the occurrence of forays. Males that forayed were more than four times as likely to sire extra-pair offspring with the female they visited compared to non-visiting males. This strongly suggests that extra-territorial forays enhance a male's extra-pair mating opportunities. Whether males copulate during such visits or whether females seek extra-pair copulations from these males remains unknown.

Key words: extra-pair behaviour, extra-territorial forays, blue tit, mating behaviour

Introduction

Monogamy was long thought to be the fundamental mating system in birds. However, since molecular techniques have become readily available, evidence has accumulated that most of the studied, socially monogamous bird species perform copulations outside the social pair-bond, often leading to extra-pair paternity (EPP) (Griffith *et al.* 2002). This genetic polygamy despite social monogamy has received much attention, because it opens up a new pathway via which sexual selection can occur through male-male competition and female choice. Much research has therefore been conducted investigating the costs and benefits of having extra-pair offspring (EPO) (Forstmeier *et al*. 2014). However, the behavioural mechanisms leading to EPP are still largely unknown.

One behavioural prerequisite for EPP is that a male and a female that do not form a social pair bond meet and copulate with each other. In territorial species, therefore, at least one of the extra-pair partners has to leave its territory to have the opportunity to interact with the extra-pair partner. Such extra-territorial movements, also termed extra-territorial forays, may serve different purposes. Firstly, individuals may leave their territory to search for food or nesting material (e.g. eastern red-winged blackbird, *Agelaius phoeniceus,* Westneat 1993; dark-eyed junco, *Junco hyemalis*, Chandler *et al.* 1997). In fact, extra-territorial movements are expected in any species that does not defend all-purpose territories. Secondly, individuals may intrude into other individuals' territories to acquire information. They may, for example, gather information about territory quality and breeding success to optimize their own reproductive decisions (e.g. collared flycatcher, *Ficedula albicollis* Doligez *et al.* 2004). Thirdly, individuals may leave their territories to actively investigate EPP opportunities. This hypothesis also received support in several species (Black-billed magpie, *Pica pica*, Buitron 1983; blue tit, Kempenaers *et al.* 1992; Wheatear, *Oenanthe oenanthe*, Currie *et al*. 1998; Superb Fairy Wren, *Malurus cyaneus*, Double & Cockburn 2000; Least flycatcher, *Empidonax minimus,* Tarof & Ratcliffe 2000; Common Yellowthroat, *Geothlypis trichas*, Pedersen *et al.* 2006; Hooded warbler, *Setophaga citrina*, Chiver *et al.* 2008). Finally, the observed patterns of extra-territorial movement may be explained by any combination of the aforementioned hypotheses. For example, an individual may foray for food outside its territory, thereby increasing its encounter rate with potential extra-pair mates and thus its probability to obtain EPP.

Most studies reporting on extra-territorial forays suffer from relatively low sample sizes (on average 26 individuals of one or both sexes observed, range: 4-99, N = 39 studies, Table S1) and discontinuous data. This is not surprising, because the data have been obtained through time-consuming direct behavioural observations or via radio-tracking. Behavioral observations are also difficult, because individuals generally appear to remain silent and behave unobtrusively during extra-territorial forays (Chaffinch, *Fringilla coelebs*, Hanski & Hanski 1988; Indigo Bunting, *Passerina cyanea*, Westneat 1988; Hooded warbler, Stutchbury 1998; Nightingale, *Luscinia megarhynchos*, Naguib *et al.* 2001; Acadian Flycatcher, *Empidonax virescens*, Woolfenden *et al.* 2005), probably to reduce the risk of aggressive encounters with the territorial owners. Currently, only one study investigated foraying behavior on a continuous basis using an automated telemetry system on a total of 21 individuals (Yellow-breasted chat, *Icterina virens*, Ward *et al.* 2014). Otherwise, no population-wide, continuous

data on extra-territorial forays are available.

Another problem with the current data is that it is usually difficult to establish a link between individual foraying behavior and EPP. In birds, copulations are often cryptic and short, and therefore extra-pair copulations are rarely observed during extra-territorial forays (for references see Westneat and Stewart 2003). A previous study on blue tits, however, found that 47% of observed extra-territorial forays by females led to interactions, sometimes courtships and rarely copulations with the resident male (Kempenaers *et al.* 1992). Because no foraging took place during such forays, the authors concluded that indeed female blue tits actively forayed into neighbouring territories to seek extra-pair copulations (Kempenaers *et al.* 1992).

In this study we used radio-frequency identification (RFID) technology to automatically record all visits of PIT-tagged (passive integrated transponder), resident blue tits to the nestboxes of other breeding pairs. Because individuals were recorded only if they were near the entrance hole of the nesting cavity, our data underestimate the total frequency of extra-territorial forays. However, visits to other pairs' nests were recorded systematically for the entire population throughout the breeding season and they are likely to have a purpose other than food acquisition. Here, we use a dataset on nestbox visitations during four breeding seasons. The aim of our study is to investigate – for each sex separately – (1) the timing of forays to other pairs' nests in relation to time of day and season, (2) characteristics of individuals that perform such forays, and (3) the relationship between the frequency of visits to other pairs' nestboxes and the occurrence of EPP.

Methods

Study area and field procedures

We collected our data in a mixed-deciduous oak forest ("Westerholz", 48°08' 26'' N 10°53' 29'' E) close to Landsberg am Lech, Germany. Since 2007, 277 small-holed nestboxes (diameter entrance hole: 26 mm, distance to nestbox floor: 16 cm) were occupied by 60 - 150 breeding pairs of blue tits each year.

During each breeding season, starting in March, nestboxes were checked at least once a week to monitor the onset and progress of nest building. Boxes were checked daily to determine the date of the first egg, clutch size, the date of first hatching, brood size, the number of fledglings, and the date of fledging.

To assess adult identity, we caught all parents when the oldest nestling was 8–12 days old; under adverse weather conditions when brood desertion due to capture is more likely, we caught only unknown parents. After capture, we banded each parent with a metal ring and three colour bands, measured tarsus and wing (3rd primary) length and body mass, took a ca. 50 µL blood sample from

the brachial vein for paternity analysis, and equipped them with a PIT-tag under the skin on the back (2010-2012: EM4102 ISO animal tag 134.2kHz ISO, 8.5 mm x 2.12 mm, 0.067 g; 2013: BIOMARK HPT8 animal tag 134.2 kHz FDXB, 8.4 mm x 1.4 mm, 0.03 g, Biomark, Idaho, USA).

Thirteen days after the first young hatched, we banded each nestling with a metal band, measured wing and tarsus length and body mass, and took a ca. 50 µL blood sample from the brachial vein.

During winter, we also caught blue tits roosting inside a nestbox, and banded, measured, blood sampled, and transpondered them. For further details on the study area and the field procedures see Schlicht *et al.* 2012.

Paternity analysis

To assess the paternity of all offspring, we compared the genotypes from parents and their putative offspring using a set of 11 microsatellite markers (PC3, PC4, PC7, PC8, PC9, Pocc1, Pocc6, PC4, Pat43, PK11, and PK12). We also determined the sex of all individuals using the P2P8 primers. For a detailed description of the paternity analysis see Schlicht *et al.* 2012 and Delhey *et al.* 2003.

Recording of nestbox visits and assessment of data reliability

All 277 nestboxes in the study area are equipped with a RFID-reader (Elatec, Eichenau, Germany) around the entrance hole, which records the identity of all PIT-tagged (transpondered) individuals that approach the nestbox entrance (roughly within 3 cm), and the date and time at which these events occur. Studies that use such technology often need to define how to separate one visit from the next, and especially a nestbox entry from a nestbox exit. Therefore, our nestboxes are also equipped with light barriers at the inside and at the outside of the entrance hole to record the direction in which the bird moves. This allows us to separate individual events with a high certainty. Additionally, most birds performed only one visit to a foreign nestbox (see below), and full certainty about the separation of individual events is therefore not crucial. For details on this automated recording system see Schlicht *et al.* 2012. For the purpose of this study, we used data recorded between 14 March and 21 May in the breeding seasons 2010-2013.

Our data suffer two main weaknesses. (1) Only birds that have a transponder are recorded. Because we only captured parents that slept in a nestbox during winter or that fed offspring, only 45% of the females (14% of 1st year breeders, N = 202, 76% of older females, N = 207) and 51% of the males (25% of 1st year breeders, N = 234; 77% of older males, N = 159) in the population carried a transponder at the beginning of the breeding season. The age bias results from the fact that adult birds have usually been transpondered in a previous breeding season. However, this should not bias our results, because we only compare the levels of EPP within the group of individuals that carry a transponder. (2) During the study period the automated nestboxes malfunctioned on average less than 2% of the

time (2010: 0%, 2011: 4%, 2012: 0%, 2013: 3%), e.g. due to low batteries. This means that individuals that visited non-functioning nestboxes were not recorded, leading to an underestimate of the true number of visits and a reduced reliability of the variable 'visits performed' (see below). However, due to the low failure rates, we do not expect malfunctioning nestboxes to produce any biases in the data.

Data selection

For most analyses, the data were restricted in two ways. (1) We excluded nestbox visits that occurred early in the season, when individuals might still be sampling suitable nest sites. Therefore, we only included visits when all individuals involved (the visiting individual and the visited pair) were settled, i.e. nest building in their respective boxes had started (at least the bottom of the box covered with moss). (2) We only included visits that were potentially relevant for extra-pair behavior, i.e. we excluded all visits that took place after the day the penultimate egg was laid by the relevant female (in case of a visiting female: this female; in case of a visiting male: the female of the visited pair). This dataset is the basis for all tables, figures, and analyses except those presented in Fig. 3. We did not restrict visits to the presumed fertile period of the involved females, because visits may be linked to EPP indirectly, i.e. not only via copulations that take place during such visits (see Discussion).

For the analysis of seasonal effects on nestbox visitation behavior (Fig. 3), we defined three periods using the day of the first egg (= day 0) as a reference point. (1) The pre-laying period (day -5 to day -1). During this period, females are expected to be fertile (e.g. Kempenaers, Verheyen, and Dhondt 1995), and it may be a critical period for extra-pair copulations in blue tits (Magrath *et al.* 2009, Vedder *et al.* 2009). (2) The early laying period (day 0 to day 4). This period encompasses on average the first half of the laying period, during which extra-pair copulations may still take place in blue tits (Magrath *et al.* 2009, Vedder *et al.* 2009) and in other species (Hoi 1997). (3) The late laying period (day 5 to day 9). Because the blue tits in our study population lay on average ten eggs, this encompasses the second half of laying, during which extra-pair fertilizations are expected to be more rare (Magrath *et al.* 2009, Vedder *et al.* 2009). Thus, for all individuals, the three periods have the same length, and visit frequencies are directly comparable. We further restricted our dataset for this analysis such that only individuals were included (1) that had settled at least five days before egg laying commenced and (2) that performed at least one extra-territorial visit during one of the periods.

Statistical analysis

For statistical analysis we used R 3.0.2 R Core Team 2013. We used the package lme4 Bates *et al*. 2013 for generalized linear mixed effects models (GLMM).

For the analysis of seasonal effects on nestbox visitation behavior, we assigned for each visiting

individual and each period (pre-laying, early laying, late laying) whether or not a visit took place (yes/no). We did not use counts of visits (or visit rates) because most individuals performed only one or few visits in total (see Fig. 1). We then used a binomial GLMM with the response variable 'visit occurred' (yes/no) and the explanatory variables 'period' for males and for females separately. We added year and identity of the visiting individual as random factors to account for dependence among data-points. We performed this procedure once using 'period' relative to the nest of the visiting birds, and once using 'period' relative to the nest of the visited birds. Finally, we used the R package 'multcomp' (Hothorn *et al.* 2008) to compare visitation probabilities during different periods and correct significance levels for multiple testing.

Female visits to other nestboxes were rare (Table S2), so we further only analysed male visitation behavior. We investigated whether age and body size influenced the likelihood that a male visited other breeding boxes. We used a GLMM with the binary ('binomial') response variable 'visits performed' (yes/no), male age (yearling or older) and male tarsus length (centred and scaled) as explanatory variables, and male identity and year as random factors. Again, we used a binary response, because most individuals only visited once or a few times.

To investigate (a) the relationship between breeding distance and visiting behavior and (b) the relationship between visitation behavior and EPP, while controlling for the known effects of male age and breeding distance on EPP, we used an approach described in detail in Schlicht *et al.* (2014) and the respective R package 'expp' (Valcu & Schlicht 2013). In this approach, the potential extra-pair partners (all male-female combinations in the population except for the social pair) are treated as individual data points in a binomial GLMM. For (a) we assigned to each male-female combination whether the specific male visited the specific female (response variable), and their breeding distance (explanatory variable). In (b) we determined for each male-female combination whether or not they had extra-pair offspring together (yes/no, response variable), and used their breeding distance (in number of territories), the age of the male, his standardized body size (divided by two standard deviations to allow comparison with two-level factors, Gelman 2008), and whether the male visited that female's nestbox as explanatory variables. In both analyses, we added 'male identity', 'female identity', and 'year' as random factors to the model to correct for pseudoreplication. Because no visits took place further than two territories apart, we further restricted our dataset to direct and second-order neighbours. For details on assessment of territory boundaries using Dirichlet tiles see Valcu and Kempenaers (2010).

Results

Frequency of extra-territorial nestbox visits

Overall, we recorded 385 extra-territorial visits by 54 males and 137 visits by 15 females during the early breeding season (before clutch completion). Individuals performed 1-91 visits to one to four different nestboxes (Fig. 1).

Seasonal and daily timing of extra-territorial nestbox visits

The distribution of male and female visits in relation to the time of day and in relation to the own or the visited pair's reproductive status is shown in Fig. 2. Males tended to perform more visits during the late than during the early laying period of their own female (multiple comparison; Estimate±SE: 1.12±0.49, z = 2.30, P = 0.06, all other P-values > 0.15), but did not adjust their visits in relation to the breeding status of the female they visited (all P-values > 0.97). Females showed no clear patterns (all P-values > 0.13, Fig. 3, Table S3). Both males and females performed most visits in the later morning (not around dawn) or in the early afternoon (Fig. 2).

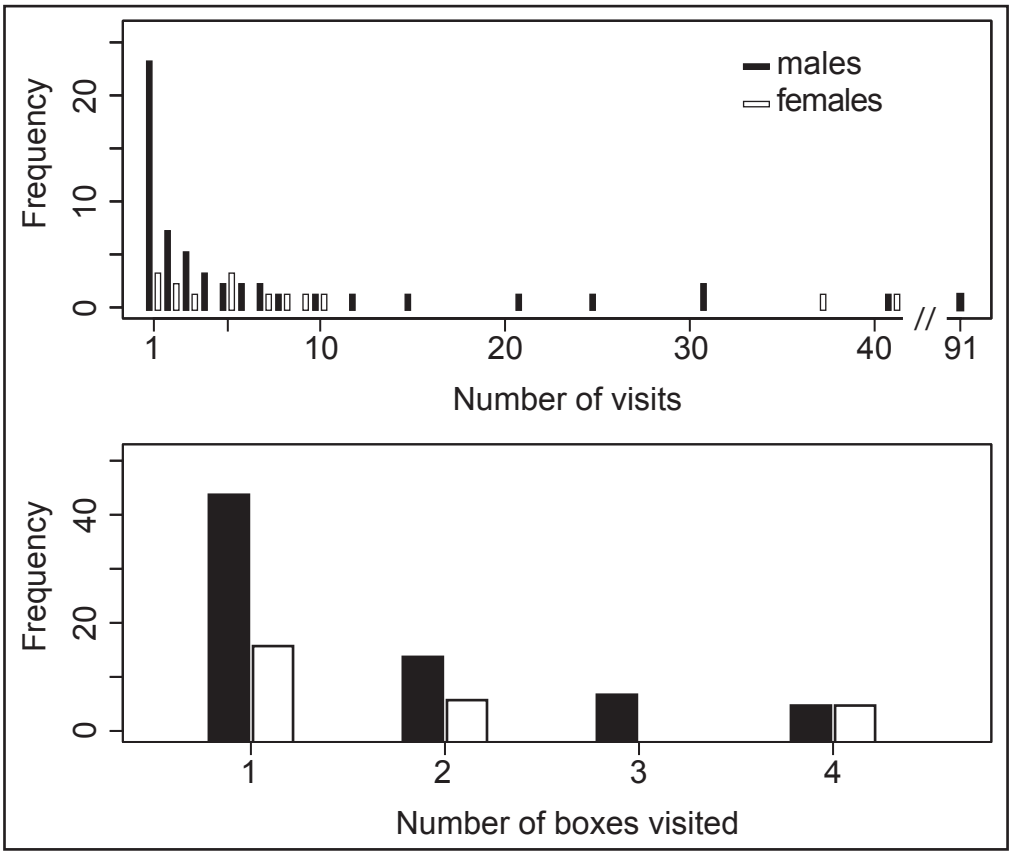

Figure 1. Frequency distribution of how often individual blue tits visited other nestboxes (upper panel) and how many different nestboxes single individuals visited during the early breeding season (lower panel). The x-axis in the upper panel is interrupted to show one male that performed a total of 91 visits to foreign boxes.

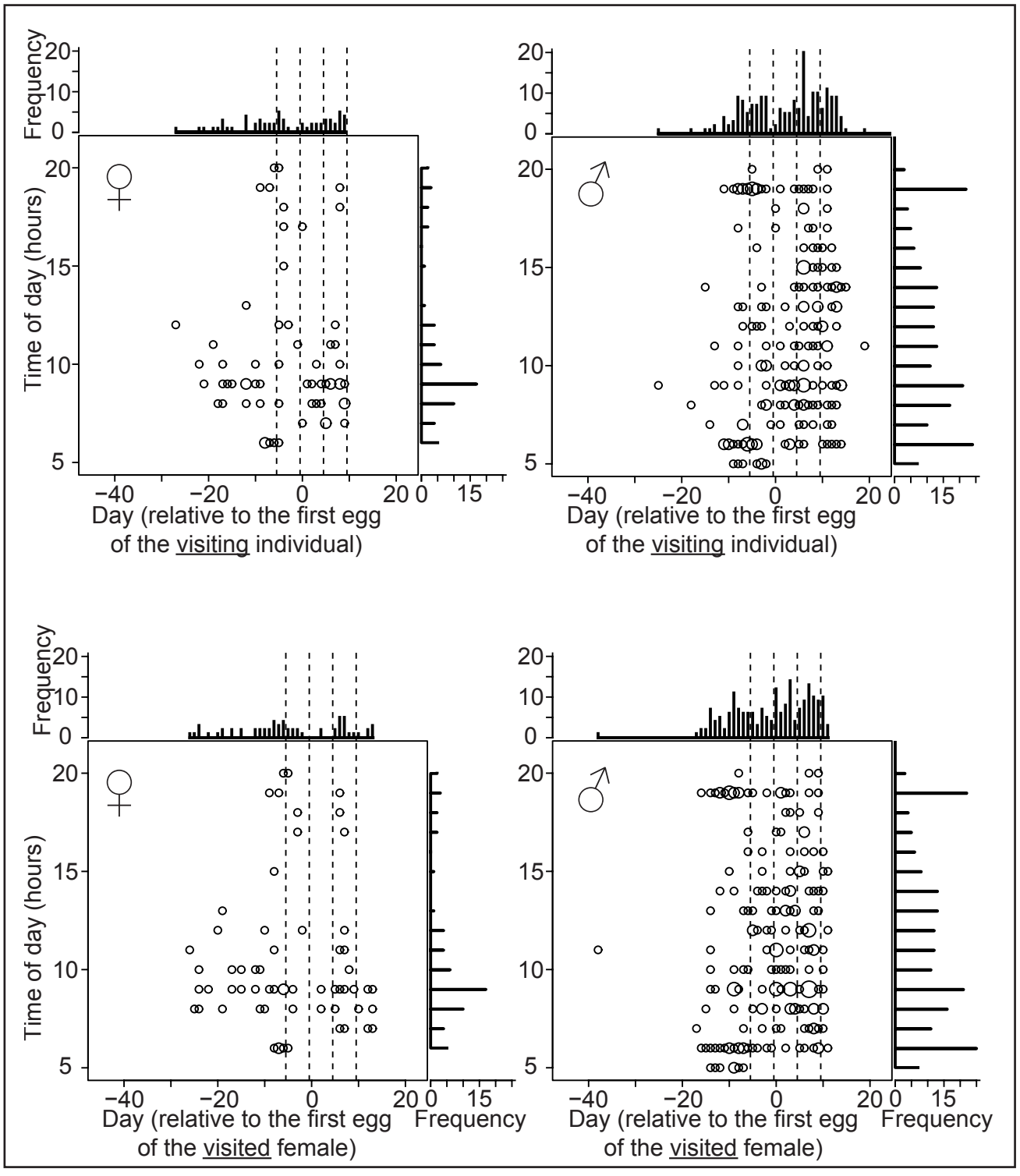

Figure 2. Distribution of blue tit visits to foreign breeding boxes throughout the day and the season. In the upper panels, 'season' is defined in relation to the first egg of the visiting individuals. In the lower panels, 'season' is defined in relation to the first egg of the visited individuals. The left two panels show female visits, the right two panels male visits. Small dots indicate one visit, large dots indicate multiple visits. The frequency distribution throughout the day and season is presented separately on the right, respectively at the top, of each panel. Dashed lines indicate the time periods used for analysis ('pre-laying', 'early laying', 'late laying'). Note that individuals may be represented in the graph multiple times.

Male characteristics and extra-territorial nestbox visits

Males were more likely to visit direct neighbours than second-rank neighbours (breeding distance: -12.84±2.48, z = -5.17, P < 0.001, $N_{\text{all male-female combinations}}$ = 3485, $N_{\text{males performing visit}}$ = 67). Larger males were more likely to perform visits (0.47±0.21, z = 2.22, P = 0.03, $N_{\text{total number of males}}$ = 225), but male age did not play a role (-0.05±0.48, z = -0.11, P = 0.91, $N_{\text{total number of males}}$ = 225).

Extra-territorial nestbox visits and EPP

Males were more likely to sire at least one extra-pair offspring with a female if they had visited her nestbox. This effect was independent of the relationship between breeding distance and male age and body size on the occurrence of EPP (Table 1, Fig. 4)

Discussion

Using four years of data, we found that many blue tits visited other pairs' breeding boxes throughout the early breeding season (after settlement, but before clutch completion). Males performed more visits than females, and mostly to neighbouring pairs. Larger, but not older, males were more likely to perform visits. Whether a male performed a visit predicted his extra-pair success with the visited female, even when controlling for confounding effects (the three most established correlates of extra-pair paternity in blue tits: male age, male tarsus length, and the breeding distance between the extra-pair partners).

Description of behavior

In early spring, blue tits are especially aggressive when defending their territories against intruders (supplement of Mutzel *et al.* 2013). Nevertheless, we found a surprisingly large number of visits to

Table 1. Results of a GLMM explaining the probability that a male-female pair has extra-pair offspring together in relation to breeding distance (neighbour order), male age, male size and male extra-territorial visits to the female's nestbox.

	baseline	level of comparison	Estimate±SE	z value	P-value
intercept			-3.86±0.39	-9.78	< 0.001
breeding distance	2nd rank	1st rank	1.31±0.24	5.54	< 0.001
male age	1st year	≥ 2nd year	1.00±0.40	2.51	0.01
male tarsus		(continuous)	0.64±0.25	2.55	0.01
male visits	No	Yes	1.55±0.41	3.80	< 0.001

Sample sizes: N = 2894 male-female combinations, $N_{\text{males performing visit}}$ = 51. The column 'baseline' indicates the factor level of each variable at which the intercept was calculated. Male body size was standardized by dividing an individual's tarsus length by two standard deviations (0.98).

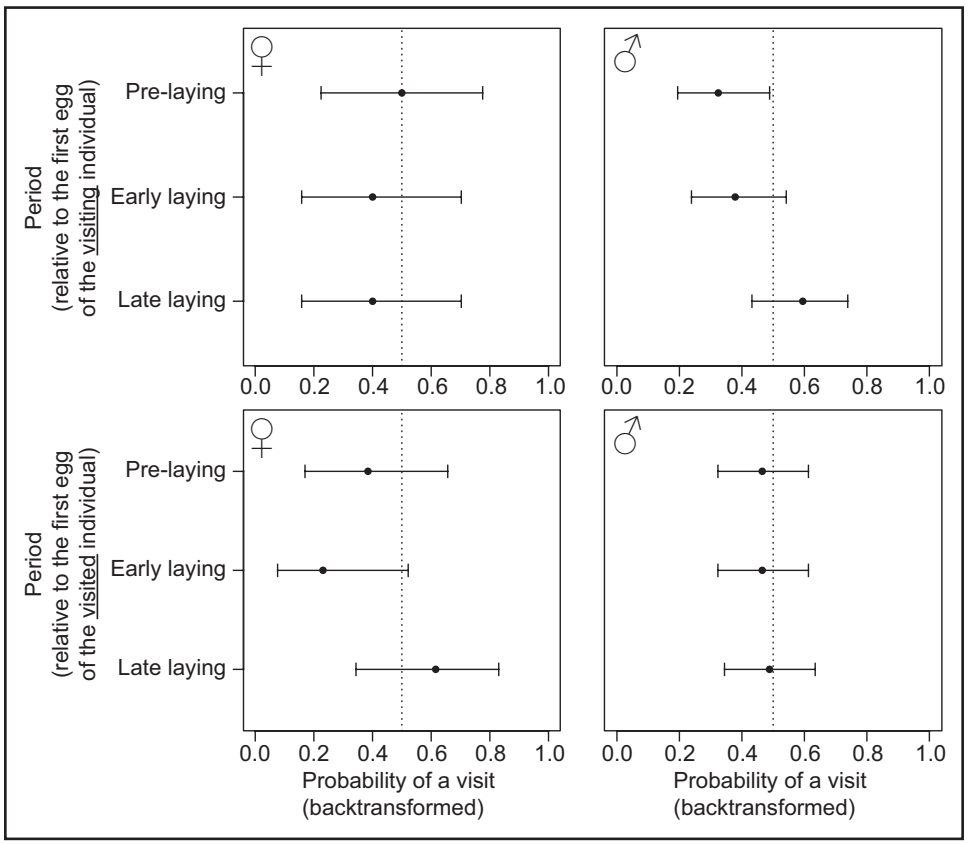

Figure 3. Probability of blue tit visits to foreign nestboxes during different periods of the breeding season. In the upper panels, the period is defined in relation to the first egg of the visiting females (left) and males (right). In the lower two panels, the period is defined in relation to the first egg of the visited individuals for female (left) and male (right) visitors. Shown are estimates and confidence intervals.

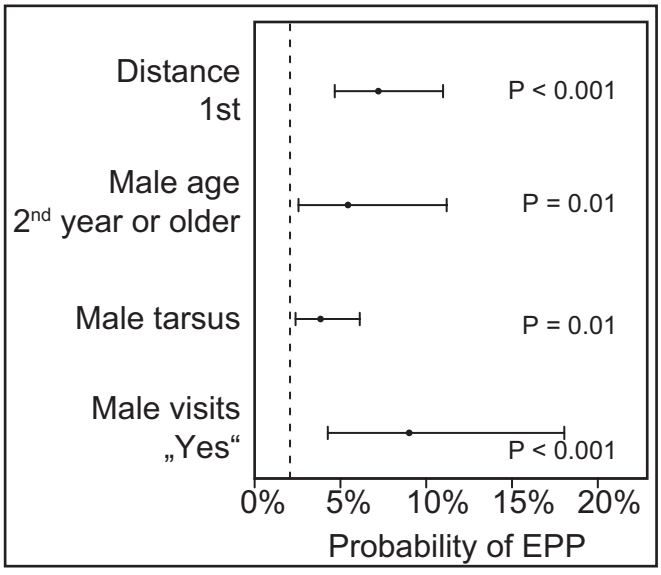

Figure 4. Effects of breeding distance (neighbour order), male age, male size, and extra-territorial nestbox visits (yes/no) on the occurrence of extra-pair paternity. Shown are back-transformed estimates and confidence intervals from the model in Table 1. The dashed line indicates the intercept, which is the baseline probability of 2% that a male sires extra-pair offspring with a particular female, given that he is a yearling (1st year breeder) and a second-order neighbour who did not perform any visit to the female's nestbox.

other pairs' nestboxes throughout the day and throughout the early breeding season. Given that nestboxes do not represent or lead to a food source, it seems unlikely that these visits were linked to foraging behavior. However, such visits clearly offer an opportunity to meet a potential extra-pair mate (Kempenaers *et al.* 1992). This is especially true for males, because females spend more time at their nest than their social mates during nest building and egg laying (Perrins 1979, pp. 156 - 160).

Only during nine of the recorded 522 visits, a visiting individual was at the box around the same time (within 30 seconds) as a resident pair member, suggesting that meetings at the nestbox are overall rare. This may indicate that the visits do not necessarily lead immediately to extra-pair mating opportunities, but may serve the inspection of potential extra-pair partners. It is important to keep in mind, however, that only individuals that approached the nestbox were recorded. Individuals that meet within the territory but not at the box itself therefore go unnoticed. Interestingly, in six out of the nine cases, a visiting male met the resident female, suggesting that males do have the possibility to meet a potential extra-pair mate during a visit.

In total, males performed more visits than females. This could be because females performed fewer forays, as reported previously for blue tits (Kempenaers *et al.* 1992), and other species (Sheldon 1994a, Marthinsen *et al.* 2005, Currie *et al.* 1998, but see Mays & Ritchison 2004). Alternatively, females may have approached the nestboxes less often during their extra-territorial visits to avoid female-female aggression.

In the context of extra-pair behavior, we expected males to perform most visits when their own female was not receptive for extra-pair copulations, to avoid losing paternity themselves (Kempenaers *et al.* 1995). In blue tits extra-pair copulations appear to take place mostly before or during early egg laying (Magrath *et al.* 2009, Vedder *et al.* 2009, see also Westneat 1987, Sheldon 1994a). Thus, we expected males to perform most visits when their own female was in the late laying period and to visit females that were in the pre-laying or early laying period. This was only partly true, as males indeed tended to perform more visits during the later laying stages of their female. They did not, however, adjust their visitation behavior to the visited female's reproductive period, suggesting either, that extra-pair copulations do not take place during such visits, or that males have incomplete knowledge about the breeding status of females other than their own mate.

If females visit extra-territorial nestboxes in search of extra-pair copulations, they should preferentially visit during their own fertile period, while the fertility status of the female whose nestbox they visit should only play a minor role. We do not have enough data to present clear evidence whether this was the case or not. Although the effect sizes contradicted the expected pattern (Fig. 3, Tables S3), drawing clear conclusions seems unwarranted at this stage.

Overall, both males and females performed most visits in the late morning until noon or in the early afternoon. This may reflect their general activity patterns; indeed, the daily patterns of visits to foreign nestboxes we observed coincide remarkably with the pattern of blue tit and great tit *Parus major* winter feeding activity (Owen 1954). Higher extra-territorial foray rates or higher extra-pair copulation rates in late morning hours were previously reported in some studies (e.g. Chaffinch,

Sheldon 1994b; Common yellowthroat, Pedersen *et al.* 2006), but not in others (e.g. Chaffinch, Hanski 1992; Indigo Bunting, Westneat 1993; hooded warbler, Pitcher & Stutchbury 2000).

Who performs visits?

In contrast to most previous studies, our sample size of male (but not female) visits allowed us to robustly test whether male characteristics determined their foraying behavior. Previous studies suggest that more competitive males, e.g. older (Indigo Buntings, Westneat 1988, Reed bunting, *Emberiza schoeniclus* Kleven *et al.* 2006, Black-billed magpie, Buitron 1983) and larger males (Hooded warbler, Stutchbury 1998) might be more likely to perform extra-territorial forays. Older and larger males may for instance be more competitive, and may therefore be more likely to risk intrusions into other pairs' territories and visit their nests, as they may suffer a smaller cost from potential aggressive encounters by the territory owners. Furthermore, previous work on blue tits showed that older and larger males were more likely to sire extra-pair offspring (Kempenaers *et al.* 1992, Schlicht *et al.* 2014). Interestingly, larger, but not older, males were indeed more likely to perform visits to other pairs' nestboxes. Visits to foreign nestboxes may therefore act as a behavioural link driving the relationship between EPP and male body size in blue tits (see below).

Extra-territorial nestbox visits predict EPP

Territorial intrusions that go to such length as to visit the nestbox itself are rare events. However, because their cause is clearly not food acquisition they may represent an active behavior that enhances extra-pair mating opportunities. For males, we had a sufficient sample size to investigate whether visiting a specific female's nestbox is related to siring extra-pair offspring with that female. We found that a male that visited another female's nestbox was indeed more likely to sire extra-pair offspring with that female than males that made no such visits, even when controlling for breeding distance and male age and body size (Fig. 4). Interestingly, the effect of male body size on EPP did not disappear when including visiting behavior, indicating that breeding distance, male age, male body size, and visiting behavior constitute independent drivers of EPP. Noticeably, the effect size for "visited nestbox" was of similar strength compared to the effect of breeding distance and male age, the best-established predictors of EPP found in the literature (Akçay & Roughgarden 2007). This suggests that the behavior of visiting another pair's nestbox has a strong influence on a male's chance to sire extra-pair offspring with that female.

There are three behavioural scenarios which could lead to such an effect. (1) Extra-pair copulations could take place during a visit. In this case EPP would directly result from the male visits, leading to the observed correlation. (2) Extra-pair copulations might take place after a visit. Males may use their visits to investigate the female breeding status or quality, or to display to females that are present, thereby increasing their opportunities for an extra-pair copulation later on. (3) Extra-pair copulations may have taken place before the visit. After an extra-pair copulation took place, a

male may check up on the breeding status of the female e.g. to investigate whether he should keep courting or not. This could for instance be the case, if some extra-pair copulations take place long before egg-laying, when a fertilization might be unlikely. If copulations take place during visits to foreign nestboxes, then visits that occur close to the peak of female extra-pair activity (assumed to be around or shortly before egg-laying, Magrath *et al.* 2009, Vedder *et al.* 2009, see also Westneat 1987, Sheldon 1994a) should be more likely to result in extra-pair offspring. Interestingly, there was no such effect in our data (effect size±SE for distance of visit to first egg on EPP: 0.01±0.04, P = 0.80), suggesting that extra-pair copulations may indeed not take place during visits to foreign nestboxes. Interestingly, only visits that took place after pairs had settled at a nestbox (as defined in Methods), but not prior visits, predicted EPP (effects size±SE for visits prior to settlement on EPP: 0.54±0.36, P = 0.13, $N_{\text{visits prior to settlement}}$ = 214; for comparison see Table 1). This indicates that the behavior we measure is specific for the period between nestbox occupation and the end of laying, and that it is independent of fixed individual characteristics, such as the overall propensity of an individual to inspect nestboxes. This suggests that visits to foreign nestboxes may be an active male behavior to increase extra-pair gains.

A previous study on blue tits suggested that EPP is female-driven, with females actively seeking extra-pair copulations during their extra-territorial forays (Kempenaers *et al.* 1992). Our results do not necessarily contradict this, for example if extra-pair copulations do not take place during male visits (see above). Even if they do, we cannot exclude the importance of female extra-territorial nest visits, because we do not have enough datapoints to analyse the relationship between female visits and EPP.

Conclusions

In blue tits, visits to the nest of other breeding pairs take place throughout the day (with a peak in the morning) and throughout the season. Larger, but not older males were more likely to visit. The large effect sizes of breeding distance, male age and body size, and male visiting behavior on the occurrence of EPP within the same model indicate that these variables represent to some degree independent mechanisms driving the patterns of EPP we observe. Specifically, male visits to foreign nests before and during egg laying seem to be an active behavior that enhances extra-pair mating opportunities.

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Chapter 5

Courtship calls in blue tits: occurrence throughout the day and season and their link to extra-pair paternity

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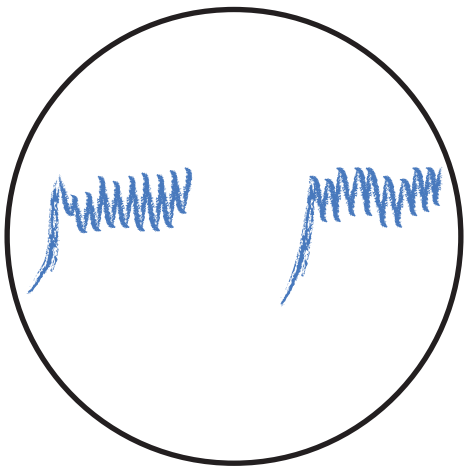

Abstract

Many arboreal songbirds perform short and cryptic courtships and copulations in thickets or dense canopy. Therefore, broad-scale behavioural information on courtships is generally unavailable. At the same time, many bird species have specific vocalizations which are used only in the context of courtships or (attempted) copulations. We therefore propose to utilize these vocalizations in order to gain information about the courtship behaviour of species where the courtships themselves are difficult to assess. We use sound recordings of blue tit courtship vocalizations with the following aims. (1) We describe the occurrence of courtship calls throughout the day and throughout the season, compare the courtship rates we find with rates in the literature, and investigate whether courtship call recordings are indeed a good proxy for overall courtship behaviour. (2) We describe the occurrence of courtship calls which are apparently not directed to the social mate, and link this behaviour to extra-pair paternity gains and losses. (3) We report on a pilot playback experiment to assess behavioural reactions of individuals to courtship calls within their territory.

Introduction

Elaborate courtship displays serving the function of mate attraction are well known in many bird species that do not form social pairs, e.g. birds of paradise (Scholes III 2008), manakins (Lukianchuck & Doucet 2014), and bower birds (Kelley & Endler 2012). However, courtship behaviour also occurs in socially monogamous species for the purpose of attracting and securing a mate (e.g. in shrikes, Tryjanowski & Hromada 2005; boobies, Torres & Velando 2005; or birds of prey, Arroyo *et al.* 2013). Additionally, courtships may serve the function of mating with an extra-pair mate (Tryjanowski & Hromada 2005). Courtship (or copulation) rates, and the timing of courtships and copulations throughout the day and season differ markedly among species (e.g. tropical birds (e.g. hummingbirds Pizo 2012) vs. temperate birds (e.g. blue tits, Kempenaers 1994), see also Birkhead & Møller 1992 p. 98). Ultimately courtship behaviour leads up to copulation and in consequence fertilization and offspring. Therefore, behavioural information about courtship and copulation behaviour is a crucial aspect in the study of mating systems. In many arboreal songbirds courtships and copulations are short, cryptic, and take place in thickets or dense canopy, so that visual observation is largely inhibited (e.g. Smith 1988). A number of these species perform specific vocalizations in the presence of a potential mating partner during courtships and/or copulations (e.g. blue tit, Bijnens & Dhondt 1984; Black-capped chickadee, Ficken 1978, Red-winged blackbirds, Grey 1996; see also Birkhead & Møller 1992 p. 85, Suppl. P1). Precise descriptions of such calls, however, are sparse. Recording courtship vocalizations could be an easy and relatively time-saving method to assess overall courtship rates in bird species where courtships are otherwise difficult to observe. They may also be used to assess individual courtship rates, if the identity of the calling individual is known. Additionally, in socially monogamous species these calls could be used to assess extra-pair courtship rates, whenever the location of one of the partners is known (e.g. roosting in a nest, or caught by an experimenter).

We use recordings of vocalizations in a blue tit (*Cyanistes caeruleus*) population to study their courtship calls. These calls have previously been described for this species as "copulation calls" (Bijnens & Dhondt 1984). To stress that these calls do not necessarily lead to a copulation (Bijnens & Dhondt 1984, pers. obs. BK and LS) we will use the term "courtship calls" throughout this study. The study has three aims. (1) To describe the occurrence of courtship calls throughout the day and throughout the season, compare the courtship rates we find with rates in the literature, and investigate whether courtship call recordings are indeed a good proxy for overall courtship behaviour. (2) To describe the occurrence of courtship calls which are apparently not directed to the social mate, and link this behaviour to extra-pair paternity gains and losses. (3) We report on a pilot playback experiment to assess behavioural reactions of individuals to courtship calls within their territory.

Methods

Study site and species

Since 2007, we monitored the breeding behaviour of cavity-nesting blue tits in a mixed-deciduous oak forest ("Westerholz", 48° 08' 26'' N 10° 53' 29'' E) close to Landsberg am Lech, Germany. The 277 small-holed nestboxes in the area (diameter entrance hole: 26 mm, distance to nestbox floor: 16 mm) are occupied by 60 to 150 blue tit breeding pairs each year. Other bird species only exceptionally use the nestboxes. Blue tits are ca. 11g songbirds. Their breeding season begins in March with territory establishment and nest building (territory establishment may start earlier; first finished nests usually present by the end of March). This is also the time period in which most social pairs form. Most females start egg-laying Mid-April, and lay one egg per day (median clutch size: 10 eggs, range: 4-16). Once the clutch is complete females incubate the eggs alone for on average ca. 13 days, so that most offspring hatch Mid-May. They are fed inside the nesting cavity for ca. three weeks, and still receive parental provisioning after fledging (Perrins 1979, pp. 163-164). After independence of the offspring (sometimes a bit earlier) the parents start the moult (Perrins 1979 p. 189). Although replacement clutches regularly occur after nest failure, we never observed any second breeding attempts (after successful fledging of the first brood).

Sound recordings throughout the day and throughout the season

To obtain a first idea about the seasonal and daily distribution of courtship calls, we used 4 sound recorders in 2013, which continuously recorded from 45 minutes before sunrise to 15 minutes after sunset from the 30th of March until the 6th of May. This period covers the fertile period of all females in the population. Based on these data (see Fig. S1), we aimed to achieve more robust estimates of courtship call rates throughout the study area and over a longer time period. To this end, we installed 16 sound recorders throughout the study area in 2014, two of which failed immediately or shortly after installation, and are thus not used for analysis (Fig. S2). The 14 remaining recorders

recorded half an hour around civic sunrise and civic sunset (starting 15 minutes before sunrise or sunset, respectively). At dawn, this is the time period when most females emerged from their roosting place (Schlicht *et al.* 2014). Additionally, at each full hour, we recorded for 15 minutes. The 14 sound recorders used for analysis started to record on the 5th of March and rendered data at least until the 10th of May. Because the sound analyses proved to be time-consuming, in this study we analysed 70% of the data for every second day to achieve valid daily and seasonal distributions. The data presented here thus result from analysis of more than 1300 hours of recordings. In all files we (1) marked all courtship calls (defined as 1 call if the syllables were less than 15 seconds apart); (2) scored rainfall (no rain; light rain; heavy rain); and (3) scored whether at least one blue tit song occurred within the same recording period (song is here defined as at least three consecutive strophes; for song description see Bijnens & Dhondt 1984). We used the latter measurement to ensure that breeding blue tits were present (see Fig. S3).

Dawn sound recordings within a nestbox

In 2012 and 2013, we recorded sounds at 59 nestboxes (37 in 2012, 22 in 2013) by placing a microphone inside the nestbox of a breeding blue tit female (this was done in the context of another study; Schlicht *et al.* 2014). These recordings took place around dawn (1 hour before to 0.5 hours after civic sunrise). This allowed us to assess whether the female was inside the nestbox (sounds of her moving around in the box) and when she left the nestbox (noise of exiting through the hole and wing-fluttering; see also Schlicht *et al.* 2014). Simultaneously, we assessed the vocal behaviour of the male outside of the nestbox.

We assume here that a male that is producing a prolonged song bout near the nestbox is the territorial male, because song playbacks at this time of the day and during this period are highly effective in attracting and inducing counter-singing and aggressive behaviour of territorial males (Mutzel *et al.* 2013). We never noticed two males singing close to the nest box at the same time.

We focus here only on the days where data is available for all males: day -5 (five days before the female lays the first egg) to day 0 (day of first egg). We only consider courtship calls to be “potential extra-pair courtships” (see below), if (1) the social female was clearly still inside the nestbox, so that a within-pair courtship could be excluded (see below), and if (2) the courtship calls occurred close in time to the focal male’s song start or end (“interrupting the male’s song”, for an example see Fig. S4). In most cases, this distinction was clear. Whenever it was unclear, we conservatively excluded the respective courtship calls from the analysis. We also excluded the rare (N = 3) courtship calls which took place directly at or potentially within the nestbox, because these are likely to be interactions with the social female.

Experiment

In 2014, we conducted a pilot experiment in which we attracted territorial males using song playback 30 minutes before sunrise while the social female was known to roost inside the nestbox (assessed via a camera inside the nestbox; see Fig. 1a). As soon as the territorial male was clearly behaviourally reacting to the song playback (approaching the loudspeaker and/or counter-singing), we played back standardized courtship calls (or silence as a control) for 1.5 minutes to investigate any behavioural response to the playback. We repeated the same procedure 30 minutes after sunrise to acquire more data (female location unknown). All behavioural observations were recorded on voice recorders (VoiceTracer, Philipps, Amsterdam, Netherlands) for later analysis. We recorded any male behaviour in connection with courtship calls both during the playback and during observation periods between the two playback sessions (distance to the nestbox and movements through the territory, singing activity, territorial behaviour (e.g. whether the attention was on the loudspeaker), and male courtship behaviour). We also noted the occurrence of courtship calls in the vicinity. In 2014 the blue tit season was exceptionally early (on average 13 days earlier than in previous seasons) and highly synchronous; most females started to lay within one week. We could therefore only obtain a total of 14 observations sessions ($N_{control}$ = 5, $N_{experimental}$ = 9). Further details on the experimental procedures can be found in the Suppl. P2. Note that most males were attracted by the song playback, indicating that the playback quality was sufficient.

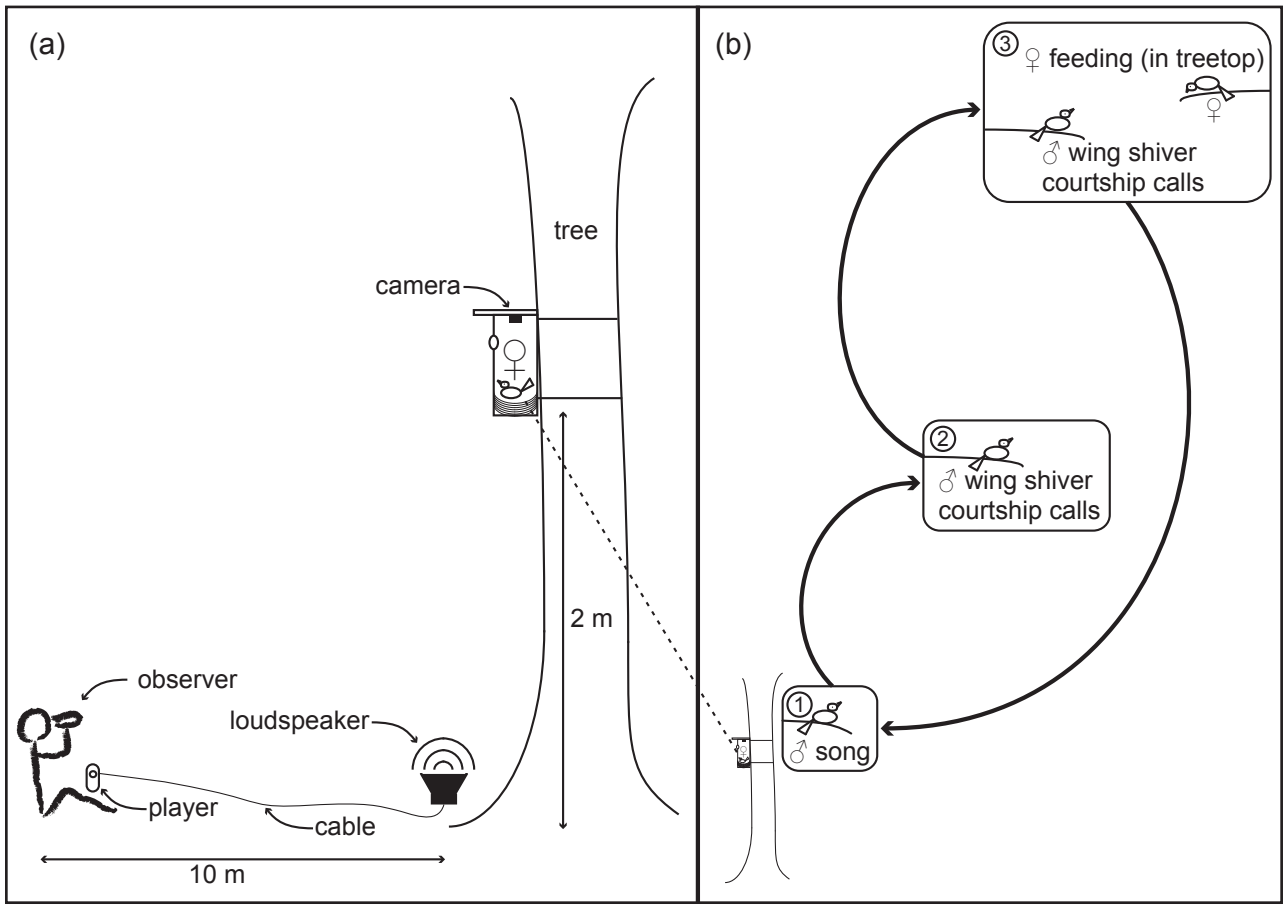

Figure 1. Diagram of (a) experimental setup and (b) observation of an extra-pair courtship. See methods and results for detailed descriptions.

Sound recordings

All sound recordings were made with SM2 or SM2+ Song Meters (www.wildlife-acoustics.de) with a sampling rate between 22500 and 41500 Hz. The sound analysis was performed visually using the software Songscope (Wildlife Acoustics Inc. 2007-2010, Concord, USA; Software version 4.1.3.A) or the software Audacity (Audacity 1999-2012 Audacity Team, Software version 2.0.2, http://audacity.sourceforge.net/). All data for daily and seasonal distribution of courtship calls was analysed by LS. This data was analysed blindly with regards to the date and the time to ensure objective analysis. Two thirds of the data on "potential extra-pair courtships" (see below) were analysed by LS (for each nestbox days -4, -3, -1, 0). The remaining data was analysed by Jonas Geurden (for each nestbox day -5 and day -2). We therefore can exclude an observer bias when comparing data from different nestboxes.

Statistical analyses

All statistical analyses were performed using the software R 3.1.1 (R Core Team 2014). We tested for a correlation between courtship frequency and extra-pair paternity (gains and losses in number of offspring; count data). We used a generalized linear models based on a negative binomial distribution to model the relationship between the number of extra-pair offspring gained (response) and the number of courtship calls (centred and standardized, fixed effect), because the models based on a Poisson distribution were highly overdispersed. Including "year" into the model did not change the results (Table S1). We used a generalized linear mixed-effect model with a binomial distribution to model the relationship between the proportion of extra-pair offspring that a male lost in his own brood (response) and the number of courtship calls (centred and standardized, fixed effect). To account for overdispersion, we included a random effect "row number" with a unique value for every row into the model (Gelman & Hill 2007). Including an interaction with "year" did not influence the estimate, but reduced the power (Table S2). To test for a correlation between the number of potential male extra-pair courtships and male age, we used a generalized linear model based on a Poisson distribution (no overdispersion) with "number of potential extra-pair courtships" as the response variable and "male age" (first year breeder or older) as the fixed effect. Including an interaction with study year did not change the estimates (Table S3). Three males were recorded in both years and therefore create a potential for pseudoreplication. Excluding these three males from the analysis in one year did not change the estimates or significance levels, and we therefore only report the model including all datapoints.

Results

Occurrence of courtship calls

Across the season, we recorded a total of 902 courtship calls in 1315 hours of data. During the peak season (1-15 April) when most females were laying eggs, the average rate of courtship calls throughout the day was 1.5 calls/hour, and around sunrise 6.3 calls/hour. The daily and seasonal distributions of courtship calls are shown in Fig. 2.

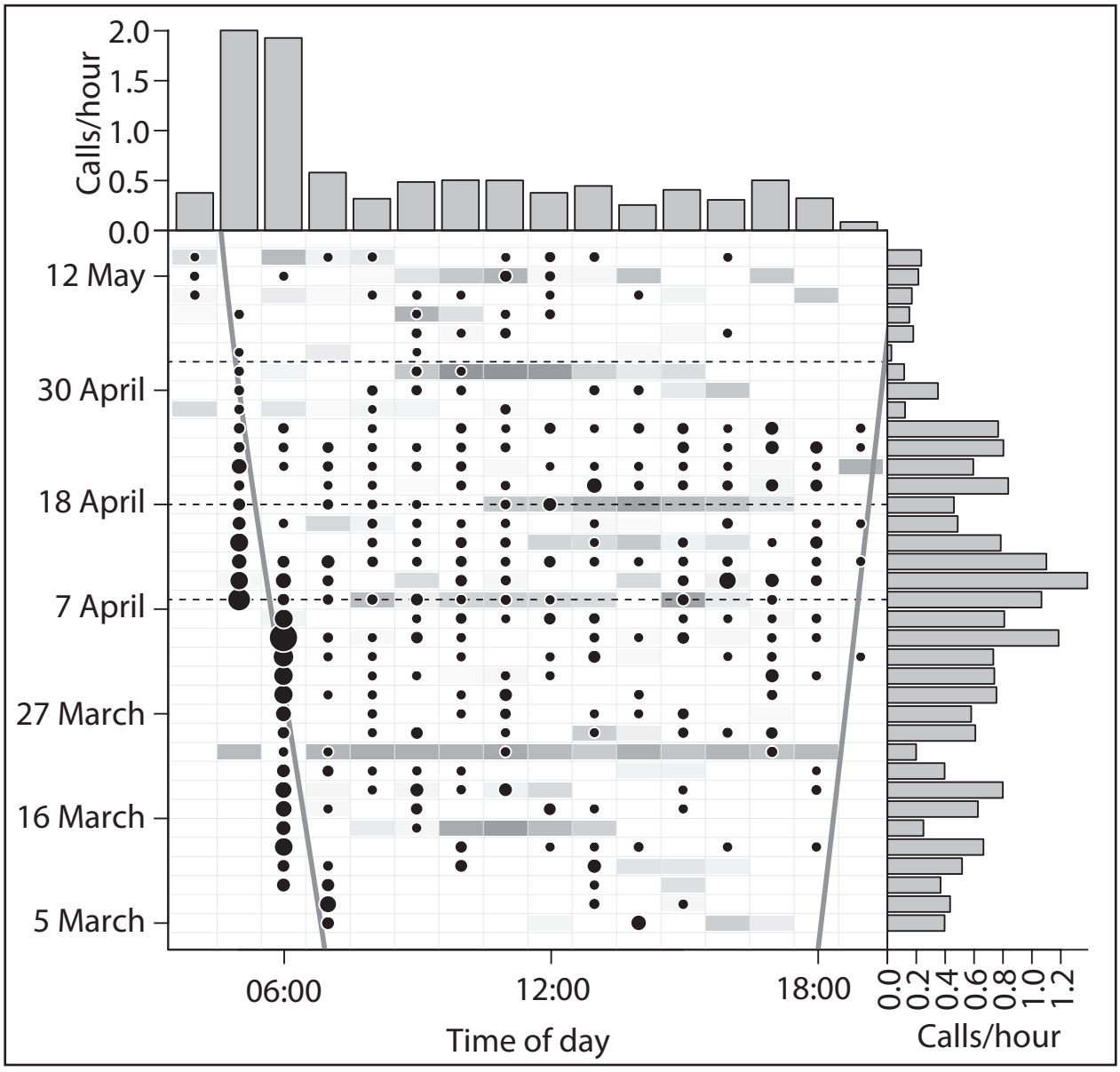

Figure 2: Daily and seasonal patterns of copulation calls. The size of the dots reflects the number of recordings where song was observed (smallest point size: 0.2 calls/hour; largest points: 10 calls/hour). Grey rectangles indicate rainfall, with dark rectangles indicating heavy rain throughout the forest (averaged over all files at the specific date and hour; rain scored as 'no rain', 'light rain': analysis of courtship calls unaffected, and 'heavy rain': analysis of courtship calls partly impaired). The two grey lines indicate civic sunrise and sunset, respectively. The three dashed lines indicate the mean laying start, mean date of clutch completion, and mean hatching date across the population in 2014. Overall densities are shown for the time of day (right) and the season (top). The axes of the density plots are scaled to show average values per hour. Note that the season was highly synchronous in 2014 (most females started egg-laying within one week).

Direct observations of "extra-pair" courtship behaviour

We describe two direct observations of possible extra-pair courtship (mate during the playback experiment). The first observation is illustrated in Fig. 1b. A male sang at his nestbox (dawn chorus). He interrupted his song, flew ca. 10 m away on a branch halfway up the canopy, and started to wing-shiver, while uttering courtship calls. After a few seconds, he moved to the top of the canopy, where another blue tit was observed, apparently foraging. The male continued wing-shivering and uttering courtship calls, while approaching this bird (presumably a female) to a distance of < 30 cm. The female did not show any obvious response to this performance, but continued foraging. After ca. 20 seconds the male stopped his courtship behaviour and flew back to the nestbox, where he resumed singing. In another case, a male interrupted his dawn song and flew ca. 20 m in a straight line producing courtship calls, but immediately returned; no other bird was seen.

Frequency of extra-pair courtships and paternity patterns

During dawn, when the females had not yet emerged from their nestbox, we observed 261 courtship calls by 59 males (mean per male: 4.4 calls, range: 1-10; data ranges over 6 days). Because these calls are used by blue tits only in interaction with members of the opposite sex, the number of these courtship events for a male could be linked to his extra-pair courtship rate (Fig. 3, see also below). However, we did not find a correlation between the number of such courtship calls observed during their mate's fertile period (day -5 to 0) and male extra-pair gains (estimate = -0.03±0.23, P = 0.91, Fig. 4a, see also Table S1). There was a borderline correlation of the number of "extra-pair courtships" with the proportion of offspring lost (estimate ± SE = -0.48±0.24, P = 0.05, Fig. 4b, see also Table S2). Older males did not produce significantly more "extra-pair courtships" than males in their first breeding season (yearling: mean±SE = 4.43±0.37, older: mean±SE = 4.7±0.33, P = 0.66, N = 28 yearling and 20 older males, see also Table S3).

Playback experiment

In the try-out session (Suppl. P2), the treated male (in the absence of his partner) immediately switched to social calls and moved in short hops through the branches close to the nestbox during the playback of courtship calls. This suggests that the male was searching for his social female. However, in the main experiment (Westerholz), we observed no such behaviour or any other behavioural changes when courtship calls were played back. Female emergence was never apparently triggered by song playback or courtship call playback.

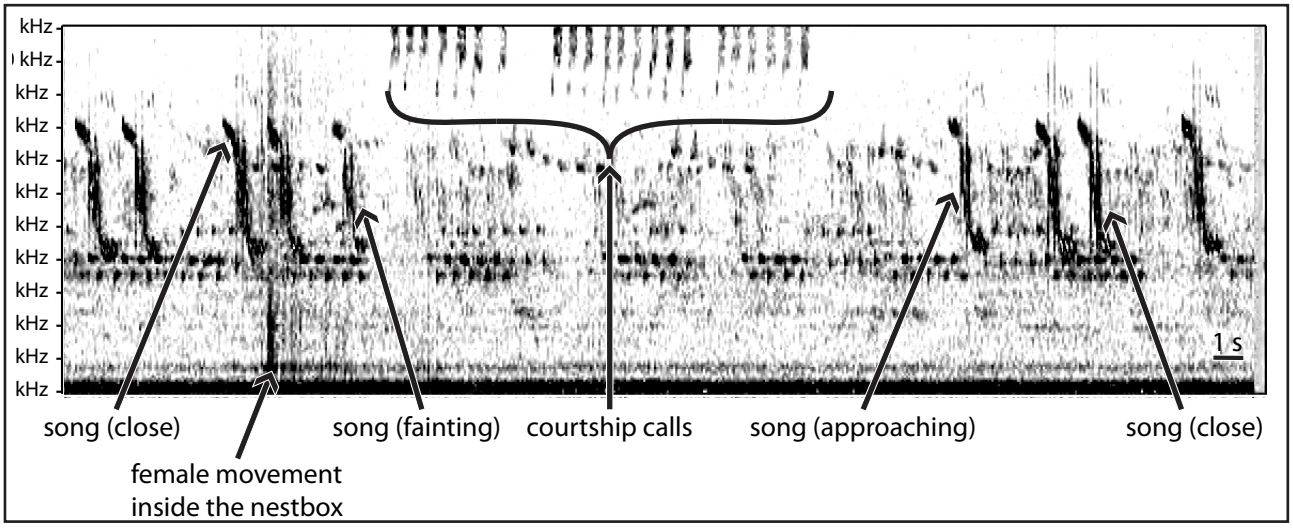

Figure 3. Typical example of courtship calls "interrupting" the focal male's song. Note that the amplitude of the song decreases immediately before the courtship calls start (indicating that the male is flying away) and increases again after the calls (indicating that the male is coming back). The female is moving inside the nestbox, and in this case clearly not the receiver of the courtship calls.

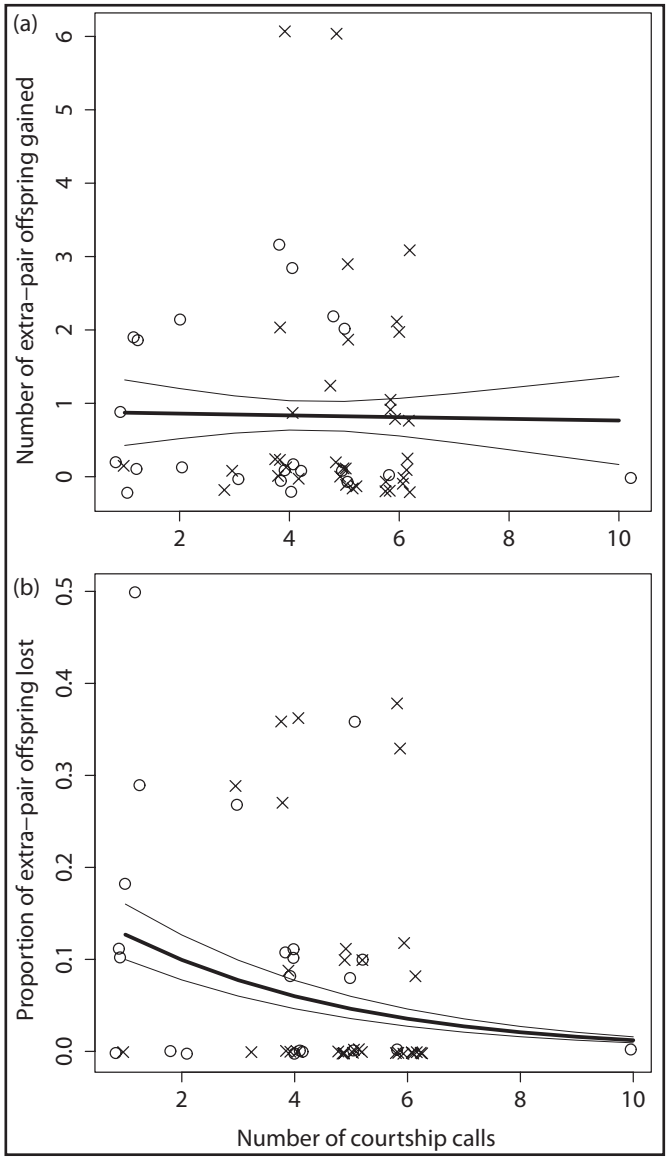

Figure 4. Relationship between number of courtship calls observed during the social mate's fertile period and (a) the number of extra-pair offspring gained and (b) the proportion of paternity lost in the own brood. Shown are jittered datapoints and backtransformed model estimates and confidence intervals. Statistical results are shown in Table S1 (N = 59). Crosses represent data from 2012, open circles data from 2013.

Discussion

We found that courtship calls occur frequently throughout the day and season, with a peak during the female laying period and at dawn. Courtship calls also regularly occurred while the social female was still roosting in the nestbox, indicating that extra-pair courtships may have taken place. This is also confirmed by an observation of an extra-pair courtship during the experimental observations. These "extra-pair courtships" were correlated with extra-pair losses, but not gains.

Description of courtship calls

We found courtship calls throughout the entire recording period (5 March to 14 May), a longer time period than previously reported (Bijnens & Dhondt 1984). During the peak season (nest-building to clutch completion) an observational study on blue tits reported 1-2 courtships (and ca. 0.5 copulations) per hour (Kempenaers 1994). We report 1.5 courtship calls per hour that we find during the peak season (1-15 April). This indicates that recording courtship calls at least in blue tits may be a good predictor of overall courtship rates. In contrast to our data, a previous study reported only a subtle peak of courtship activity at dawn (Kempenaers 1994). However, this study focused on observations of birds with known identity, which is difficult to assess with the low light levels available before sunrise. Blue tits often court or copulate immediately after the female emerges from her roosting place (Poesel *et al.* 2004, our unpublished data). Additionally, previous evidence suggests that extra-pair behaviour may take place during dawn (Poesel *et al.* 2006, Kempenaers 1994). Relatively high numbers of courtship calls around sunrise therefore concur with previous findings.

"Extra-pair courtships" and paternity gains

Courtship calls are a means of communication among opposite-sex blue tits (Bijnens & Dhondt 1984). All males recorded in the study area also produced at least one potential "extra-pair courtship" in the early morning before their social female emerged from her nestbox. Indeed, we observed one presumed extra-pair copulation in 2014 (Fig. 1b). It did not lead to a copulation, as the female showed no obvious reaction and continued foraging. Nevertheless this observation shows that at least some of the courtship calls we recorded are likely to represent extra-pair courtships. Interestingly, the male interrupted his courtship after ca. 20 seconds, and returned to his song-post before the female left the territory, indicating that males do not generally harass females that pass through the territory incessantly. Interestingly, the number of "extra-pair courtships" of a male was not correlated to his extra-pair paternity gains. This may have several reasons.

Firstly, copulations may take place outside of the time interval during which we recorded. A previous study in blue tits found a higher rate of extra-pair courtships in the early morning (Kempenaers 1994), and our correlational data suggests that there is a peak of copulation activity in the morning.

Recording in the morning should therefore capture meaningful events. Nevertheless, behaviour important in the context of EPP also occurs throughout the day (Kempenaers *et al.* 1992, Schlicht *et al.* 2014 *in prep.* (Chapter 4 of this thesis)). Additionally, if males have EPO with asynchronous females, e.g. due to a trade-off between mate-guarding and investing into EPP, the time period right before the egg laying of the social female may not be representative. However, breeding synchrony does not seem to influence EPP in blue tits (Kempenaers 1997, Schlicht *et al. under review* (Chapter 2 of this thesis)).

Secondly, by definition a male's chances for the "extra-pair courtships" we recorded are limited by the time at which his social mate emerges from the nest. This means that we did not observe all males the same amount of time. However, female emergence is likely to be a biologically meaningful terminator of male extra-pair behaviour at this time of the day, as the male often ceases his singing activity at female emergence and leaves together with the female to forage (Poesel *et al.* 2004, our data on early morning song).

Thirdly, the majority of extra-pair courtships may not lead to copulations. In the case of female choice, a high number of courtships by an unattractive male will not necessarily increase his chances to sire EPO. Interestingly, we found no difference in the number of potential extra-pair courtships between males in their first season and older males, although older males are more likely to acquire extra-pair offspring in this and other blue tit populations (Schlicht *et al. under review* (Chapter 2 of this thesis), Kempenaers *et al.* 1997), and across species (Akçay & Roughgarden 2007). Potentially our data therefore suggest that in blue tits mate choice may be of importance.

Finally, the majority of extra-pair copulations may not lead to fertilizations. This could occur for instance due to male-specific copulation abilities (e.g. experienced males are better at copulating successfully with females, reported by Yasukawa 2013), or due to sperm competition with the within-pair male. In the latter case, however, due to sperm senescence (Møller *et al.* 2009) older males would be expected to perform worse than younger males in the context of extra-pair paternity. The opposite is the case. Alternatively, if post-copulatory (cryptic) female mate-choice is strong (as reported for hihis *Notiomystis concta* by Brekke *et al.* 2011), the predicted relationship between courtship call rate and EPP is not expected to occur. Up to date little is known about the potential for such post-copulatory processes.

Overall these data tentatively suggest that extra-pair courtship rates may not directly be linked to extra-pair gains. Assuming that our data capture relevant extra-pair courtships, this may suggest that pre- or post-copulatory mate choice may play a large role in blue tits.

"Extra-pair courtships" and paternity losses

A positive relationship between the number of extra-pair courtships and paternity losses is expected, if there is a trade-off between investing in the social partner versus investing into EPP, or if males follow a best-of-a-bad-job strategy (and invest into courtship even if they have no chance; suggested

for mate-guarding by Kempenaers *et al.* 1995). Interestingly however, we found a borderline significant effect that males that produced overall more courtship calls lost less paternity. Similar to previous studies (Kempenaers *et al.* 1995, Schlicht *et al. under review* (Chapter 2 of this thesis)) this argues against a male trade-off between investment into within-pair versus extra-pair paternity, and instead suggests that females mated to males with a high number of "extra-pair courtships" may be relatively faithful to their mates. Such a correlation could be driven for instance by a correlation between male quality and male courtship behaviour, if females produce a lower number of extra-pair offspring when mated with high-quality mates as has been reported for blue tits (Kempenaers *et al.* 1997, but see Johannessen *et al.* 2005). On the other hand the correlation may arise, if males that perform a higher number of extra-pair courtships also have high within-pair courtship and copulation rates which may act as an alternative paternity guard (Birkhead & Møller 1992 pp.152-154).

Behavioural implications

In the experimental try-out-session, we found that one male switched to social calls (in the absence of another blue tit) at the playback of courtship calls. However, we did not observe this or other changes in behaviour during the standardized playbacks. This suggests that courtship calls may not be used to eavesdrop on and interrupt the partner's copulations, although at least males would benefit from interrupting any extra-pair copulations of the own social female. Similarly to the observations from our sound recordings in 2012 and 2013, female emergence was not induced by the playbacks. It is important to keep in mind that a female does not immediately suffer from extra-pair copulations of her social mate, but only if a second female settles within the territory. Courtship calls occurred ubiquitously throughout the first half of the breeding season. It could therefore be that courtship calls are too ubiquitous to pose robust signals. On the other hand, if courtship calls are highly individual-specific, no clear reaction to our playback situation may be expected. From this pilot experiment we can tentatively conclude that there are no striking ubiquitous behavioural reactions of territorial blue tits to the courtship calls of unknown conspecifics.

Conclusions

In blue tits, where courtships and copulations are overall short and cryptic, courtship call rates corresponded overall well to courtship rates described previously in the literature. We therefore conclude that sound recordings of courtship calls are a good proxy for overall courtship rates in blue tits. Using these courtship calls, we find higher courtship rates around the egg-laying period of the females and at sunrise, but not at sunset. We find that courtships which are apparently not directed to the social female regularly occur, and are correlated with extra-pair losses, but not gains. This may tentatively suggest that male quality and pre- or post-copulatory extra-pair mate choice may be of importance in blue tits. Although males could use courtship calls as a signal to find potential extra-pair females or to interrupt extra-pair courtships of the social female, in a first pilot experiment we

find no evidence that this is the case. Overall, we believe that the study of courtship calls could lead to a better understanding of the timing and the behavioural mechanisms underlying the mating systems of birds with cryptic and short courtships and copulations.

Acknowledgments

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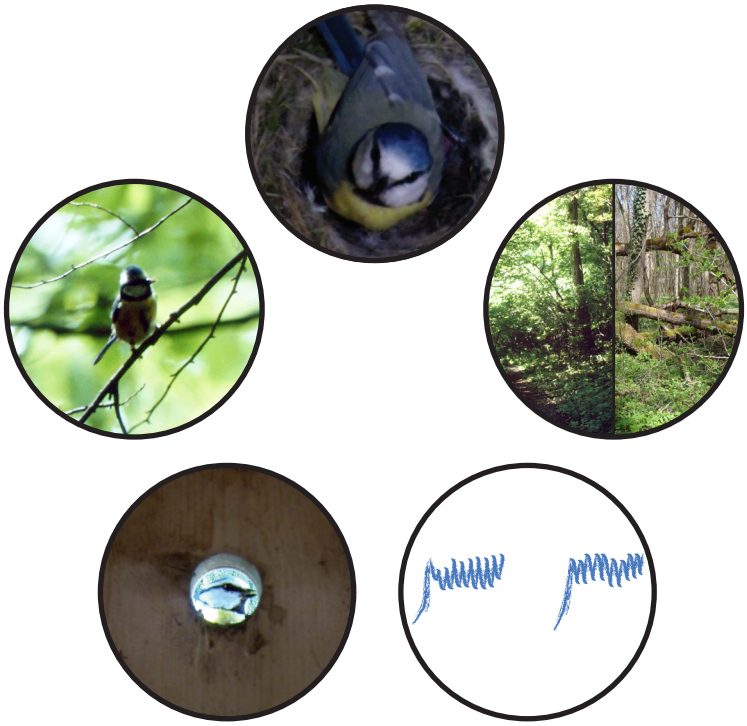
General Discussion



The studies of the previous chapters jointly explore the ecological and social setting of extra-pair paternity in blue tits. They aim to improve our understanding of how patterns of extra-pair paternity, assessed via parentage analysis, arise. Specifically, the studies combine to clarify temporal, spatial, and social constraints acting on extra-pair behaviour at the level of the species, population, and individual. Based on this information, they then go on to assess the importance of particular traits and behaviours leading to the production of extra-pair offspring.

Seasonal timing (Chapter 1, Chapter 2, and Chapter 4)

A first step to assess the seasonal timing of (successful) extra-pair events is to investigate whether extra-pair offspring are positioned randomly within the clutch. In several species, this has been investigated with mixed results (Table 1). As a general pattern, extra-pair offspring are reported early in the laying and hatching order in blue tits and in other species (Table 1). The same appears to be true in the population of blue tits from this study (Kim Teltscher, *pers. comm.,* see also Schlicht *et al.* 2012).

Potentially such an effect could arise through cryptic female choice. This would be adaptive if extra-pair offspring are of higher quality than within-pair offspring, and especially if the "advantage of an early start" (Fig. 1) is beneficial to extra-pair offspring in particular (Schlicht *et al.* 2012). It is unlikely

Table 1. Literature on a non-random distribution of extra-pair offspring throughout the laying and/or hatching order. "NA" indicates that values could not be assessed. Studies are ordered by increasing brood size.

Species	Sample size: $N_{laying\ order}$ $N_{hatching\ order}$	Brood size: Mean ± SE (range)	Extra-pair offspring	Reference
House martin (*Delichon urbica*)	6 broods 6 broods	3.36 ± 0.21 (2 – 5)	Late	Riley *et al.* 1995
Red-winged blackbird (*Agelaius phoeniceus*)	ca.119 young ca.119 young	3.43 ± 0.17 (2 – 4)	Random	Westneat *et al.* 1995
Western bluebirds (*Sialia mexicana*)	41 young 41 young	4.13 ± NA NA	Early	Ferree *et al.* 2010
House sparrow (*Passer domesticus*)	24 broods NA	5.00 ± 0.15 (3 – 7)	Early	Cordero *et al.* 1999
Tree swallow (*Tachycineta bicolor*)	22 broods 14 broods	5.8 ± 0.19 (3 – 7)	Random	Barber & Robertson 2007
Collared flycatcher (*Ficedula albicollis*)	≤ 37 broods ≤ 37 broods	NA (4 – 8)	Early	Krist *et al.* 2005
Blue tit (*Cyanistes caeruleus*)	55 broods 43 broods	11.5 ± 2.1 NA	Early	Magrath *et al.* 2009

Fig. 1. The benefit of being early. The two nestlings shown above are from the same brood and differ in size due to a pronounced hatching asynchrony. Hatching order is closely linked to laying order. (Photo courtesy of Emmi Schlicht)

that such cryptic female choice leads to a placement of extra-pair offspring in early laid eggs for two reasons. Firstly, the support for a difference in quality between extra-pair and within-pair offspring has been studied in detail, but with inconclusive results across species (Griffith *et al.* 2002, Akcay & Roughgarden 2007, Forstmeier *et al.* 2014). Differences in offspring quality are therefore unlikely to select for differential offspring paternity with laying or hatching order across species. Secondly, if extra-pair offspring are indeed of higher quality, females would be expected to produce as many extra-pair offspring as possible, and to stop producing extra-pair offspring only when extra-pair sperm becomes unavailable. It appears unlikely that females are constrained by sperm availability in the number of extra-pair young they produce, if on the other hand their cryptic choice is effective in favouring differential paternity. One male ejaculate should in principle provide sufficient sperm for fertilization of all ova. Still, the relative abundance of extra-pair sperm is an interesting aspect when considering the distribution of offspring throughout the laying order. The reported order of extra-pair offspring early in the laying and hatching sequence could indicate that in these species, the ratio of extra-pair sperm (vs. within-pair sperm) is higher during early egg laying. This suggests that the relationship of within-pair and extra-pair mating changes with the approach of the laying start. However, most species reduce or terminate copulation behaviour after the first egg is laid (Birkhead & Møller 1992, p. 97). In this case, the last eggs are usually fertilized by sperm stored from copulations several days previously at least. Then, it is difficult to see how a paucity of extra-pair sperm could lead to the strong biases in fertilization success for extra-pair sperm for early and within-pair sperm for late eggs. On the other hand, some species, including house sparrows (Møller 1987) and blue tits (Vedder *et al.* 2010), do not reduce their overall copulation behaviour during egg-laying. Instead, an experiment performed by Vedder *et al.* 2010 indicates that female blue tits may reduce their extra-pair behaviour over the course of egg-laying, while continuing copulation with their social mate throughout the entire fertile period. In blue tits, it is therefore plausible that the rareness of extra-pair copulations later during egg-laying results in a decrease in the proportion of extra-pair sperm, which in turn reduces number of extra-pair offspring in eggs late in the laying

sequence. Why extra-pair behaviour decreases during egg-laying is still unclear. As explained above, an adaptive value is difficult to envisage. Current hypotheses try to explain the patterns as a by-product of the general reproductive biology of the female. Vedder *et al.* 2010 propose a proximate mechanism whereby female hormone levels and incubation behaviour are changed in response to the contact with the eggs while roosting. Such hormonal changes are known to occur as a preparation for upcoming parental behaviour (Angelier & Chastel 2009) and may reduce extra-pair mating behaviour. Alternatively, if extra-pair behaviour is likely to occur in the morning (see below), female extra-pair behaviour may be inhibited when females emerge later from the roost during egg laying (Chapter 3) due to the time needed for laying an egg. It is yet unclear when the fertile period of a female bird starts, as sperm storage tubules in the reproductive tract allow females to store sperm over several days or even weeks (Birkhead & Møller 1992, p. 63). However, sperm that enter the female reproductive tract later are likely to have a competitive advantage over sperm from earlier copulations ("last sperm precedence", Birkhead & Møller 1992). Extra-pair behaviour in blue tits is therefore expected to be most successful if it takes place close to the respective female's laying start.

In Chapter 4, I show that blue tits frequently visited other pairs' breeding sites during the early breeding season, and find that for males this behaviour is correlated with the occurrence of extra-pair offspring with the female they visited. Similar to a previous study, males were more likely to visit other breeding sites than females (Kempenaers *et al.* 1992), limiting the sample size and power to detect the analogous effect for females. Indeed, female visits to other nesting sites could well be correlated to their extra-pair gains as well, because female foray rate in a previous study predicted female extra-pair paternity patterns (Kempenaers *et al.* 1992). Interestingly, I found for neither males nor females a clear relationship between the visitation rates and the extra-pair female's assumed fertility, i.e. the likely time window for extra-pair copulations. Males therefore apparently did not time their visits in relation to the visited female's laying start, and also did not adjust their visits in relation to their social female's laying start. For females, there is again little power to detect any effects due to the limited sample size. It is important to note here that male visits predict extra-pair gains, but only if they take place after the male was known to have its own nest (Chapter 4). This suggests that although the behaviour under study is not timed to the laying start of the visited female, it is specific to the seasonal pre-laying and laying period of the study population. Whether copulations occurred during such visits remains unexplored. Males may also visit nestboxes of foreign breeding pairs in order to gather information about the local female's breeding stage to later target these females for extra-pair copulations (e.g. completed nest without eggs may induce courtship behaviour), but this is not supported by the data. If this were the case, visits should occur before the extra-pair female's egg-laying period, while I find a weak effect in the opposite direction. In our study population, egg laying of females takes places relatively synchronously (usually most females start to lay within 11 days). A temporal adjustment of male extra-pair behaviour to the specific female they court may therefore not be necessary, because most females are available for extra-pair copulations at approximately the same time. Temporal adjustment still may occur on a coarser scale, which is not apparent when females are laying synchronously.

As a second aspect of the seasonal timing of extra-pair behaviour, it has often been proposed that if extra-pair behaviour involves male investment, males may face a trade-off during the fertile period of their mate between investing into or guarding the social female and investing into extra-pair courtships. Therefore, two individuals may be more likely to have extra-pair offspring if their respective breeding attempts are relatively asynchronous. A previous study reports that such a trade-off may not generally occur in blue tits (Kempenaers 1997). In Chapter 2, I could replicate this finding: males (females) were not more likely to have extra-pair offspring with females (males) that bred relatively asynchronously. Yet again, analysis on a population breeding less synchronously might have revealed effects on a coarser scale.

In conclusion, based on their biology blue tits are predicted to time their copulations such that they take place shortly before or during early egg laying. While this was the case on the population level, I found no evidence that birds adjust their extra-pair behaviour to this period individually, at least for males. For females the sample size is still insufficient to draw final conclusions (Chapter 4). I also did not find indication of a trade-off for males between mate-guarding and investing into extra-pair paternity (Chapter 1, Chapter 2).

Fig 2. Light conditions at sunrise when females emerge from the roost. Note the light inside the nestbox which was installed as part of the experiment presented in Chapter 3.

Daily timing (Chapter 3, Chapter 4, Chapter 5)

Many avian species show diurnal patterns in their copulatory behaviour, with most copulations occurring after the laying of the egg ("insemination window hypothesis", Birkhead & Moeller 1992 p. 97). Blue tit females lay the eggs early in the morning, usually before emerging from the roost (Perrins 1979, p. 157), and a previous study on blue tits reports slightly elevated courtship rates in the early morning, with a drop of courtship activity in the afternoon (Kempenaers 1994). The period around sunrise (Fig. 2) when females emerge from their roost may therefore also be a period during which extra-pair courtships are likely to occur. This is supported by a study reporting that males which joined the dawn chorus early were more likely to gain extra-pair offspring (Poesel *et al.* 2006), and by a second study that reported that eight of twelve observed extra-pair copulations occurred during the first hour of observations around dawn (Kempenaers 1994). I therefore hypothesized that a large proportion of the extra-pair behaviour generally occurs early in the morning.

I found mixed support for such importance of dawn behaviour on extra-pair paternity. When I investigated courtship calls rates, I found as predicted a clear peak at dawn (Chapter 5). Additionally, I recorded a high number of courtship calls which were apparently not directed immediately towards the social female, and which are likely to be extra-pair courtships. This suggests that extra-pair courtships do occur in the morning in our population, and this could well be true for blue tits in general. Extra-pair courtship rates are probably following the temporal patterns present for overall courtship rates. In Chapter 3, I also found evidence that the early morning may influence extra-pair paternity rates. I found that females that I experimentally supplied with additional light and which therefore emerged earlier from their roosting place in the morning differed from control females in their probability of producing extra-pair offspring. However, the effects went into opposite directions in the two years of the study, and the behavioural mechanism and environmental conditions that created this effect remain unexplored. Still, I can exclude emergence times as a single, general factor controlling extra-pair behaviour. This is interesting, because earlier emergence in the morning lead to desynchronized male mate-guarding behaviour (Chapter 2, data not shown), and therefore gave the female more time for extra-pair behaviour during a potentially critical time period. In addition, in Chapter 4 I found that although visits to foreign nestboxes predicted extra-pair paternity, most of them did not occur during the dawn period. Hence, previous findings reporting a peak of extra-pair behaviour in the early morning are not contradictory, but appear to be incomplete. My results indicate that behaviours which are important for extra-pair paternity are not restricted to the dawn period.

Summarizing the results on daily timing, I found in accordance with previous studies that extra-pair courtships did occur (at least partly) at dawn (Chapter 5), and that an experiment taking place at dawn influenced extra-pair paternity levels of females (Chapter 3). On the other hand, behaviours that take place throughout the day also predicted extra-pair paternity (Chapter 4). Both extra-pair behaviour at dawn and throughout the entire day are influential and probably interact with each other in determining the resultant patterns of extra-pair paternity.

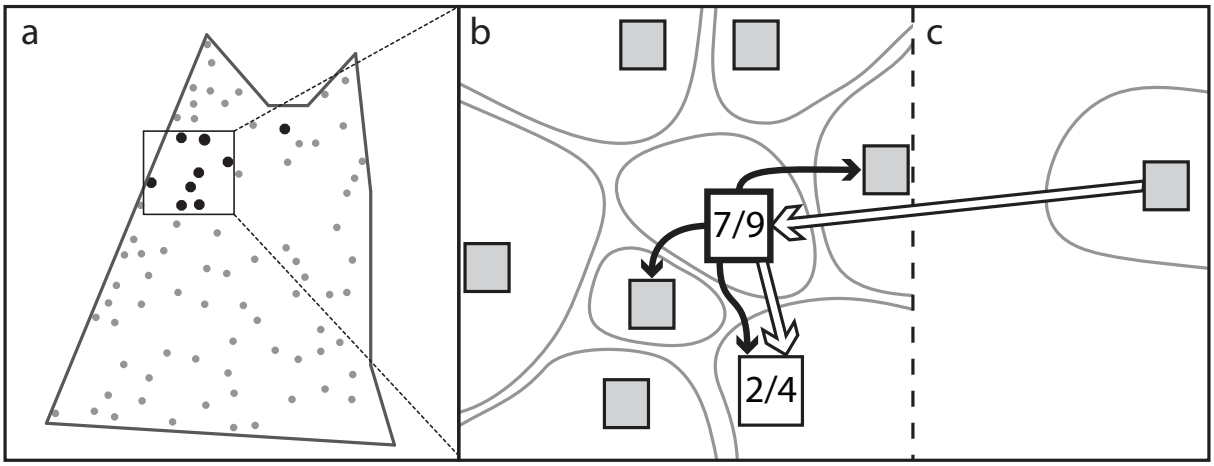


Fig. 3. Snapshot of visitation behaviour and extra-pair paternity for an example male in 2010. Squares/circles indicate the locations of breeding attempts. **(a)** shows all breeding attempts in the study area. Nestboxes with breeding attempts used in **(b)** and **(c)** are marked black. **(b)** zooms in on a subset of nestboxes. Territory borders are illustrated schematically by grey lines. White squares indicate nests where the focal male sired any offspring, either as the within-pair male (thick border) or as an extra-pair male (thin border). The number of young the focal male sired in each nest given the brood size is shown inside the squares (young sired/brood size). Straight open arrows point from the nest of an extra-pair male to the nest where he sired extra-pair offspring. Curved solid arrows point from the nest of the focal male to the nests he visited. **(c)** The breeding event separated by the dashed line is located outside of the area of focus.

Location (Chapter 4, Chapter 5)

In territorial species, at least one of the extra-pair partners has to leave the territory in order to meet a potential extra-pair mate. In most species, both sexes appear to preform forays (reviewed in Westneat & Stewart 2003). This is also true in blue tits, and of twelve observed extra-pair copulations, nine (75%) occurred on the female and three (25%) on the male territory (Kempenaers *et al.* 1992, Kempenaers *et al.* 1995). Interestingly, in the same studies only the female behaviour was correlated to extra-pair paternity levels (Kempenaers *et al.* 1992, Kempenaers *et al.* 1995). In accordance with these results, I found frequent courtships of males that were presumably aimed at extra-pair females passing through their territory (Chapter 5). Interestingly, however, the number of these courtships of a male was not correlated with his extra-pair gains. This study does therefore not imply that copulations also take place in the male's territory. On the other hand, I found that males frequently visited the nestboxes of females other than their social mate (Fig. 3), and that this behaviour strongly predicted his extra-pair success. Although it remains unknown whether copulations took place during such visits, this study presents evidence that behaviour relevant for extra-pair paternity also takes place on the female's territory.

Taken together, I found evidence that both the male's and the female's breeding location play a role in extra-pair behaviour. Again, the two effects probably interact in determining patterns of extra-pair paternity.

Is the behaviour of one sex more important than that of the other? (Chapter 3, Chapter 4, Chapter 5)

Species differ in whether the male, the female, or both actively pursue extra-pair copulations

(reviewed by Westneat & Stewart 2003). In blue tits, female behaviour was reported to influence extra-pair paternity; female foraying behaviour predicted extra-pair paternity patterns and all twelve observed extra-pair copulations were preceded by female solicitation displays and female courtship calls (Kempenaers *et al.* 1992, Kempenaers *et al.* 1995). At the same time, the same studies report that three of twelve (25%) of the copulations occurred on the female territory, which indicates a territorial intrusion by the male, and potentially active male extra-pair behaviour. The current literature therefore suggests that in the blue tit male behaviour plays a role in creating the opportunity for extra-pair copulations, but eventually it is the female behaviour that is decisive in driving the observed paternity patterns.

The importance of female behaviour is supported by three Chapters. In Chapter 2 I found that the effects of male age and body size on extra-pair paternity gains decreased with increasing distance between the potential extra-pair mates. Taken alone, the effect of age and size means that there is a difference of a certain value in paternity gains between young and old males, and between large and small males. Now including distance in our examination, I find that this value changes with the distance between two mates. Distance obscures the age and size differences between males and therefore, to receive the same paternity gains, a male must be more superior in age or size over the resident males for more distant nests. Distance should not obscure the age or size differences between males, if older or larger males intrinsically invest more into extra-pair behaviour. However, it should do so, if females have imperfect information about males breeding farther away limiting their assessment when choosing an extra-pair partner. Hence, this finding may indicate a role of female choice in determining the patterns of extra-pair paternity.

In Chapter 3, I experimentally manipulated the females, but not their mates, and found that this treatment in both years altered the observed extra-pair paternity. As noted previously, the effects went into opposite directions in the two years of the study, although the experimental procedure was strictly replicated. This suggests that the ecological or social setting has interacted with the treatment, thereby leading to differences between the two years. Which factor exactly has caused the reversion in the results is impossible to pinpoint with data for two study years only (which differ in a multitude of aspects). Nevertheless, the fact that I could experimentally alter patterns of extra-pair paternity by solely manipulating the female indicates that female behaviour is an important factor in driving the observed extra-pair paternity patterns.

In Chapter 5 I found that females were apparently often courted when moving through other territories in the early morning. This has two major implications. First, it suggests that females forayed into other territories, stressing the importance of female behaviour. Second, males regularly interrupted their dawn song for courtships, indicating that male behaviour may also be important. Interestingly, however, I found no relationship between the number of extra-pair courtships registered for a male, and his extra-pair gains. Hence, it appears that males may frequently court extra-pair females in the early morning, but may be unsuccessful in doing so. At the same time, males with more registered extra-pair courtships were less likely to lose paternity. This suggests that females monitor their partner's behaviour in relation to other females and, based on this information, engage in extra-pair

behaviour. Chapter 5 therefore confirms previous results for the blue tit that investment by both males and females influences patterns of extra-pair paternity, with the female behaviour being more important in driving these patterns. In addition, the results of Chapter 5 provide information on the mechanism leading to extra-pair behaviour of a female. Female behaviour may modulate patterns of extra-pair paternity in the absence of mate choice, for example by altering encounter rates. A female may foray into a territory with good food supplies in order to forage there, which as a by-product could increase her number of extra-pair copulations, because it increases her chances to meet and mate with the resident (successfully outcompeting, potentially attractive) male. Alternatively, a female may foray into the territory of an attractive male in order to copulate with this male (female choice). Generally, these two mechanisms cannot easily be separated. However, if female behaviour influences patterns of extra-pair paternity as a by-product of another behaviour, a high number of male "extra-pair courtships" would indicate a high number of events during which such a side-effect could manifest itself. Without female extra-pair behaviour directed towards specific males, males with more "extra-pair courtships" should therefore also have a higher level of paternity gains. I find no evidence for such a correlation. This may tentatively suggest a role for female choice of extra-pair males in blue tits.

Considering the influence of male behaviour on patterns of extra-pair paternity in more detail, in Chapter 4 I found that males visiting the breeding boxes of other pairs during the early breeding season were more likely to have extra-pair offspring with the female whose nestbox they visited (Chapter 4). Where, when, and how visitation leads to copulation is entirely unclear, but this result provides evidence that not only female behaviour, but also male behaviour may be directed towards specific partners, actively driving the observed paternity patterns. The current sample size is still limited. In the future, these data can be used to investigate within the same model the relative importance of male and female visits to foreign nestboxes on the observed extra-pair paternity patterns. This may help to understand the role of male extra-pair behaviour directed at females and female extra-pair behaviour directed at males as drivers of the observed patterns of extra-pair paternity.

In conclusion, I could verify that female behaviour can be an important factor in driving extra-pair paternity. At the same time, I also found strong evidence that male behaviour is influential and may be more important than previously thought, in particular when considered in concert with female behaviour.

Who mates with whom? (Chapter 1, Chapter 2, Chapter 4)

Many previous studies have separately investigated which males or which females have extra-pair offspring. However, extra-pair paternity results from an interaction between (at least) two individuals, which interact with each other on a spatially restricted scale (e.g. Kempenaers *et al.* 1992). The need of including these aspects into the study of extra-pair behaviour has long been stated (Westneat & Sherman 1997, Westneat & Stewart 2003). Nevertheless, this issue has not received much attention,

in part because it requires explicit modelling of a behaviour involving at least two individuals who interact in a spatially restricted framework. This is methodologically not straightforward. In Chapter 1, I therefore first validate a method to define the spatial setting in which extra-pair paternity occurs. In Chapter 2 I then propose and implement a novel approach to investigate which individuals mate with each other outside the pair-bond. I consider all potential male-female combinations that could have extra-pair offspring with each other as individual data-points. This allows including characteristics of the respective male, the respective female, and their social mates into the model. At the same time, information on the spatial setting of this specific potential extra-pair is taken into account. This approach has the advantage (a) that it allows to address not only realized, but also possible extra-pair matings, (b) that it follows closely the biology of extra-pair behaviour as an interaction between at least two individuals, and (c) that it can easily be applied to datasets from any territorial species. I use this approach to investigate which from a set of nine parameters were the most important determinants of which individuals had extra-pair offspring with one another. In accordance with previous studies on blue tits and other species (e.g. Kempenaers *et al.* 1992, Foerster *et al.*2003, Perreault *et al.* 1997), I find that neighbouring individuals were more likely to have extra-pair offspring, and that older and larger males were more likely to gain paternity. Interestingly, both effects decreased with increasing distance between the partners, potentially indicating female choice (see above). The hypothesis that the broods of the extra-pair male and the extra-pair female should be relatively asynchronous has often been put forward with mixed support (e.g. Griffith *et al.* 2002, Westneat & Stewart 2003, Taff *et al.* 2013, but see Kempenaers 1997). Results of Chapter 2 do not support that blue tit males or females have extra-pair young with especially asynchronous extra-pair mates (Fig. 4). In Chapter 4, I apply my approach to the data on visits to other pairs' breeding nestboxes. I show that a male that visits a specific nestbox is more likely to have extra-pair offspring with the female breeding there.

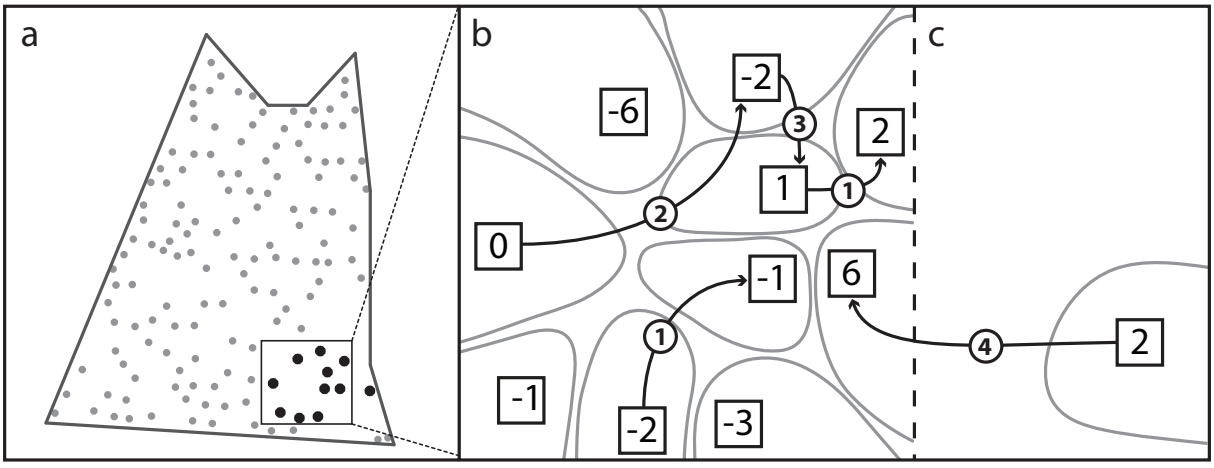


Fig. 4. Snapshot of breeding asynchrony and extra-pair paternity in an example subset of the population in 2012. Squares/circles indicate the locations of breeding attempts. **(a)** shows all breeding attempts in the study area. Nestboxes with breeding attempts used in **(b)** and **(c)** are marked black. **(b)** zooms in on a subset of nestboxes. Territory borders are illustrated schematically by grey lines. The numbers in the squares show the day the first egg was laid ("fertility peak") in relation to the 15th of April. Negative numbers therefore refer to early nests, positive numbers refer to late nests. Two nests are relatively synchronous, if the first eggs were laid close in time (e.g. one day apart). Curved arrows point from a male that sired extra-pair offspring to the nest he sired extra-pair offspring at. Numbers on arrows indicate the breeding asynchrony of two nests as the difference of the respective first egg dates in days. **(c)** The breeding event separated by the dashed line is located outside of the area of focus.

Considering the question of who mates with whom, my overall findings are: (a) individuals breeding closer together are more likely to have extra-pair offspring with one another, (b) extra-pair mating partners are not particularly asynchronous in their breeding attempts and (c) if the male visits a female's nestbox in early spring, this male and female are more likely to have extra-pair offspring with one another.

Conclusions and outlook

This study aims to further our understanding of patterns of extra-pair paternity by investigating the temporal, spatial, and social setting of extra-pair behaviour. I assessed temporal aspects both on a broader (seasonal) and smaller (daily) scale. I also examined the influence of absolute (territory ownership) and relative (distance) spatial structure. I inspected how much the males and females of extra-pair couples contribute to driving patterns of extra-pair paternity and did this including and excluding the social environment.

Altogether this study confirms findings from previous studies (e.g. the importance of female behaviour, Chapter 3), but also adds new facets (e.g. the importance of male behaviour, Chapter 4) to our understanding of extra-pair paternity in the blue tit. Results indicate that indeed the social and the ecological surroundings influence an individual's extra-pair behaviour (Chapter 2). Two of the most interesting findings are the absence of a link between the number of extra-pair courtships of a male (during the time-frame of consideration) and his extra-pair gains (Chapter 5), and the weakening of the well-established effect of male age (and body size) on extra-pair paternity with increasing distance between the extra-pair mates (Chapter 2). These two results tentatively suggest a role of female extra-pair mate choice in blue tits.

Studies using molecular data on parentage as well as considering behavioural information are necessary to understand extra-pair paternity, both from a mechanistic and from an evolutionary perspective. Selection on extra-pair behaviour occurs in a temporal, spatial, and social setting that can have profound consequences for adaptation. While more details about the influence of this setting are required, two avenues for continuing the research presented here appear particularly promising.

First, I propose to study courtship calls as a measure of courtship rates (Chapter 5). In blue tits and many other small songbirds, courtships are extremely difficult to observe directly. At the same time this is, in the behavioural sequence (see General Introduction Fig. 1), one of the most relevant traits regarding the manifestation of extra-pair paternity. While molecular data provide the better fitness estimates, they also comprise information arising from a combination of behavioural, post-copulatory, and post-fertilization processes (General Introduction Fig. 1). Successful extra-pair behaviour may thus not find a reflection in the number of offspring, if fertilization is prevented by other mechanisms. To not just understand extra-pair paternity as an avenue of reproduction, but to understand the suite of extra-pair behaviours itself, estimates of courtship rates are therefore of

high interest. As I show, they may provide information about behaviour directly linked to extra-pair copulations.

Second, I propose continued application of the approach implemented here, to study the complete set of potential extra-pair couples (including unrealized pairings, Chapter 2) instead of individual extra-pair gains or losses, and to do this while taking into account the spatial scale at which extra-pair paternity occurs (Chapter 1, Chapter 2). This may help to further disentangle the influence of the social, environmental, or individual characteristics (Chapter 2) as well as interactions among the individuals involved (Chapter 4) on patterns of extra-pair paternity we observe. This will allow us to penetrate deeper the phenomenon of extra-pair paternity in the future.

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Supplementary Material to Chapter 1

Thiessen polygons as a model for animal territory estimation

Lotte Schlicht, Mihai Valcu, and Bart Kempenaers

IBIS (2014), 156:215-219

Supplementary material for 'Thiessen polygons as a model for animal territory estimation'

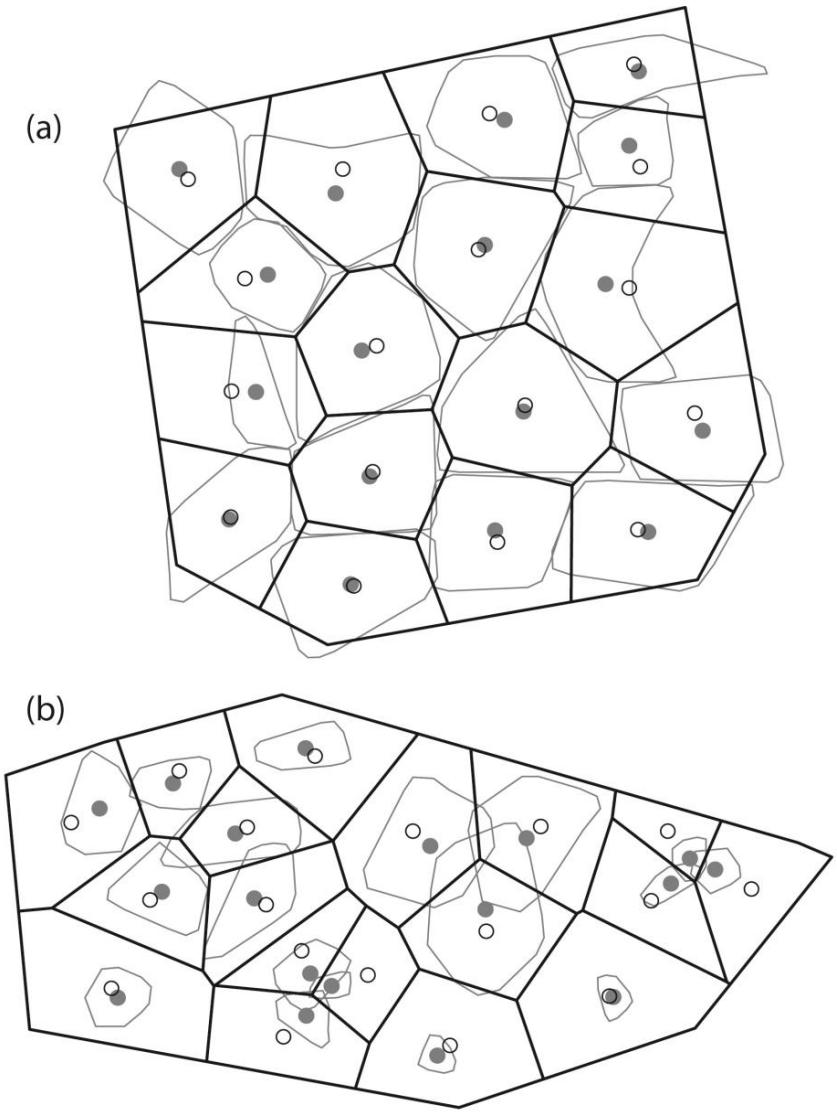

Figure S1. Visualization of mapped territories (grey) vs. Thiessen polygons (black). As an example, we devised mapped territories that resemble natural settings and parameter estimates similar to the studies (a) Pedersen 1984 and (b) Davies and Hartley (1996). Note that the mid-points of the mapped territories (filled circles) that are used as focal points to construct the Thiessen polygons do not generally coincide with the respective Thiessen polygon centroids (open circles). The focal points (e.g. nest sites) therefore do not normally lie in the middle of the respective Thiessen polygon.

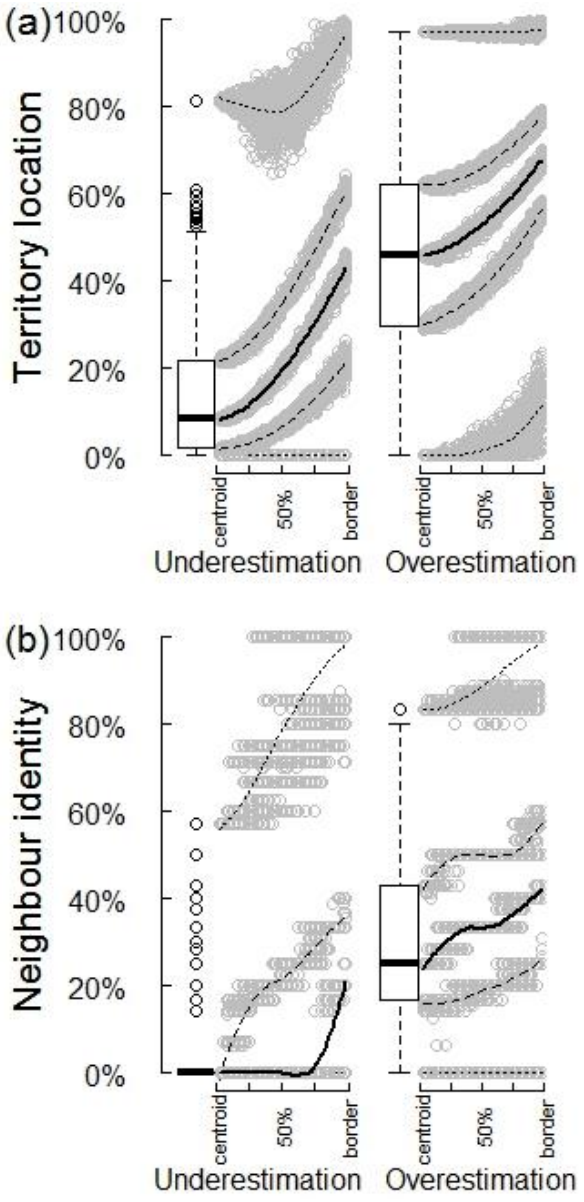

Figure S2. Underestimation and overestimation of the Thiessen polygons regarding (a) territory area and (b) neighbour identity in relation to the distance of the focal point to the respective centroid. 'Underestimation' is the percentage of the mapped area (or neighbours) that was not assigned to the corresponding Thiessen polygons. 'Overestimation' is the percentage of area (or neighbours) of the Thiessen polygon that did not overlap with the corresponding mapped area (or neighbours). Shown are boxplots based on all territories (N = 423) from the 14 studies, and the changes of the medians, first and third quartiles and the maxima and minima depending on whether the focal point used for the construction of Thiessen polygons was close to the centroid of the mapped territory or close to its border.

Supplementary Material to Chapter 2

Spatial patterns of extra-pair paternity: beyond paternity gains and losses

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Journal of Animal Ecology (2014), *accepted for publication*

Table S1. Descriptive parameters of the two blue tit study areas. 'Range' refers to the range of yearly means. The P-value is either based on a non-parametric Wilcoxon rank sum test (Test statistic: Wilcoxon) or on a Fisher's exact test (Test statistic: Fisher).

	Kolbeterberg			Westerholz			Test Statistic	P-value
	Mean ± SD	Range	N[5)]	Mean ± SD	Range	N[6)]		
Number of breeding pairs	85.6±22.3	51 - 114	7	85.4±16.3	62-114	5	Wilcoxon	0.88
Territory size (m^2) [1)]	7502±1670	5194 - 10182	430	5145±1177	4149 - 7113	401	**Wilcoxon**	**< 0.01**
Breeding asynchrony [2)]	5.04±1.36	3.26 – 6.83	5034	5.00±1.00	4.00 – 6.00	5276	Wilcoxon	0.23
Lay date (first egg) [3)]	12±3.7	5 - 16	599	17±5.0	13 - 23	427	**Wilcoxon**	**< 0.001**
Clutch size	11.1±0.5	10.6 – 11.9	596	10.4±0.4	9.9 – 10.8	427	**Wilcoxon**	**< 0.01**
Brood size [4)]	10.3±0.2	10.1 – 10.6	525	9.7±0.6	8.9 – 10.3	395	**Wilcoxon**	**< 0.001**
% broods that contained EPY[5)]	57%±5%	49% - 63%	561	46%±8%	38% – 56%	396	**Fisher**	**< 0.001**
% EPY with known sire	77%±10%	63% – 90%	907	92%±7%	82% – 99%	411	**Fisher**	**< 0.001**
% adult (vs. yearling) males	51%±9%	40% – 64%	399	55%±10%	39% – 65%	327	Fisher	0.37
Male tarsus length (mm)	17.18±0.15	17.07 – 17.50	310	16.95±0.15	16.74 – 17.09	256	**Wilcoxon**	**< 0.001**

[1)] Territory size was estimated using Thiessen polygons (see Methods).
[2)] Breeding asynchrony is defined for all pairs of broods as the difference between laying starts in days (see Methods).
[3)] Days relative to the first of April are shown (1 = 1. April).
[4)] Maximum number of hatched young in nests where at least one egg hatched.
[5)] Only clutches where at least one egg or nestling genotyped. This is in contrast to the main text, where all broods were used to insure validity of territory sizes.
[6)] Sample sizes differ because different units were used for analysis (breeding seasons, adult blue tits, potential EP-mates, individual broods, or nestling blue tits) and because not all parameters were known for all individuals.

Table S2. Test for spatial autocorrelation of the presence of unknown extra-pair fathers. We used the "Moran's I"-Index calculated in the r-package 'spdep'[1)], which takes values between -1 and +1. A positive / negative value means that data points in spatial proximity are more / less similar than expected by chance. We calculated P-values using a Markov-Chain-Monte-Carlo algorithm with 10000 iterations. Significant effects are in bold. We also calculated the assortment coefficient for unknown extra-pair sires for each year using the r-package 'assortnet' with slight modifications[2)]. We used whether or not two individuals were direct neighbors as the association matrix. In contrast to the Moran's I Index, the assortment coefficient is a measure for clustering of a trait or variable in social (or in this case: spatial) groups. The assortment coefficient ranges between '-1' (fully dis-assorted) to '1' (fully assorted). The number of unknown EP sires was estimated as the number of broods with unknown EP sires (assuming all young with unknown father within a brood were sired by the same male; minimum value) and as the number of young with unknown sires (assuming each young with unknown father was sired by a different male; maximum value). We did not calculate test statistics for 2011, because only one extra-pair sire was unknown.

Season	Moran's I	P-value (Moran's I)	Assortment coefficient ± SE	Number of known sires	Estimated number of unknown sires (min - max)	% unknown sires (min - max)
1998	-0.20	0.95	-0.02 ± 0.03	28	14 - 36	0.33 - 0.56
1999	-0.01	0.41	-0.03 ± 0.02	45	11 - 31	0.2 - 0.41
2000	-0.06	0.64	-0.02 ± 0.02	39	12 - 23	0.24 - 0.37
2001	0.05	0.17	-0.03 ± 0.02	49	7 - 11	0.12 - 0.18
2002	**0.17**	**0.01**	-0.03 ± 0.02	**68**	**26 - 63**	**0.28 - 0.48**
2003	0.05	0.18	-0.03 ± 0.02	61	21 - 37	0.26 - 0.38
2004	0.06	0.17	-0.02 ± 0.02	45	19 - 34	0.3 - 0.43
2007	-0.02	0.45	-0.03 ± 0.02	38	11 - 17	0.22 - 0.31
2008	-0.03	0.46	-0.03 ± 0.03	34	4 - 6	0.11 - 0.15
2009	0.12	0.05	-0.03 ± 0.03	36	3 - 3	0.08 - 0.08
2010	0.10	0.08	-0.03 ± 0.03	39	6 - 8	0.13 - 0.17
2011	NA	NA	NA	38	1 - 1	0.03 - 0.03

[1)] Bivand, R. (2012) spdep: Spatial dependence: weighting schemes, statistics and models. R-package version 0.0-46, URL: http://CRAN.R-project.org/package=spdep

[2)] The function 'assortment.discrete' was adjusted to ignore missing values; Reference: Farine, D. (2013) assortnet: Calculate the assortativity coefficient of weighted and binary networks. R-package version 0.1, cited in Farine, D. (2014) Measuring phenotypic assortment in animal social networks: weighted associations are more robust than binary edges. *Animal Behaviour*, 89: 11-13

Table S3. Correlation coefficients of variables initially considered for inclusion in the model to explain variation in the occurrence of extra-pair paternity. Values above the diagonal are Pearson correlation coefficients, below the diagonal are the partial correlation coefficients from the linear mixed-effect model. Correlations above 0.5 are shown in bold (see Methods for details). Please note that the male symbol for male age and male tarsus length refers to absolute values.

		Distance class	Male age		Number of neighbours		Territory size		Male tarsus length		Asynchrony	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Distance			-0.01	0.00	0.00	0.00	-0.02	-0.02	0.00	0.00	0.00	0.00
Male age	♂	-0.03		**0.92**	-0.01	-0.01	0.05	0.02	0.05	0.04	0.00	0.02
	♀	0.04	**-0.85**		-0.01	0.00	0.04	0.00	0.04	0.04	0.00	0.02
Number of neighbours	♂	0.35	0.00	-0.02		-0.01	0.22	-0.04	0.04	0.05	0.00	-0.03
	♀	0.02	-0.07	0.8	-0.06		-0.04	0.25	-0.01	0.00	-0.03	0.00
Territory size	♂	-0.08	0.04	0.01	-0.23	0.07		0.06	0.02	0.03	0.00	-0.11
	♀	0.05	0.03	-0.03	0.09	-0.25	-0.19		-0.02	0.00	-0.11	0.00
Male tarsus length	♂	-0.03	0.10	-0.08	0.00	-0.02	0.01	0.05		**0.94**	0.00	0.00
	♀	0.02	-0.09	0.08	0.00	0.02	-0.03	-0.05	**-0.84**		0.01	0.00
Asynchrony	♂	-0.01	0.01	-0.01	-0.01	0.00	-0.06	0.13	0.00	0.00		0.34
	♀	0.00	-0.02	0.00	0.01	0.00	0.10	-0.05	0.01	0.00	-0.36	

Table S4. Comparisons of the full model with all models where one parameter was removed. Models are arranged by their corrected AIC values (AICc), with the best fitting model on top. As a rule-of-thumb, a delta AICc larger than 2 indicates a better fit of the models above. Significant parameters are in bold.

Excluded parameter		No. parameters	AICc-value	Delta AICc
Full model		16	3060.53	0.00
Interaction (male age)		15	3062.52	1.99
Interaction (male body size)	♂	**15**	**3064.25**	**3.72**
Asynchrony	♀	**13**	**3065.02**	**4.49**
Number of neighbours	♀	**13**	**3065.63**	**5.10**
Male body size		**13**	**3367.05**	**6.52**
Asynchrony	♂	**13**	**3067.11**	**6.58**
Territory size	♂	**13**	**3067.84**	**7.31**
Territory size	♀	**13**	**3069.15**	**8.62**
No. neighbours	♂	**13**	**3089.55**	**29.02**
Male age		**13**	**3131.50**	**70.96**
Breeding distance		**13**	**3378.05**	**317.52**

Table S5. Relationship between probability of an event in a dataset, sample size, and power in a binomial model. We produced three simple datasets with a binomial response (here: EPP), and one binomial explanatory variable (here: male age), where the effect of the explanatory variable on the response is kept constant (binomial estimate from each model = -4.39). The datasets are summarized below as contingency tables. The datasets are devised such that for each pairwise combination of datasets only one of the following three variables differs: Number of 'rare'-events, proportion of 'rare'-events, or sample size. Dataset 1 and Dataset 2 thus have the same number of 'rare'-events, Dataset 1 and Dataset 3 have the same proportion of 'rare'-events, and Dataset2 and Dataset 3 have the same sample size. The example shows that the power of the binomial model (here estimated by z-values) increases both with the number of 'rare'-events and with the sample size, but not with the proportion of 'rare'-events. In our analysis the 'rare'-event is 'EPP yes', the 'frequent'-event is 'EPP no'. The explanatory variable in the analysis could be e.g. 'male age' (1st year breeder vs. older)

		Explanatory variable (e.g. male age)								
		Dataset 1			Dataset 2			Dataset 3		
		1st year	older	SUM	1st year	older	SUM	1st year	older	SUM
Response variable (e.g. EPP)	'frequent' event, e.g. no EPP	10	90	90	90	810	900	50	450	500
	'rare' event, e.g. EPP yes	90	10	90	90	10	100	450	50	500
	SUM	100	100	180	180	820	1000	500	500	1000
% 'rare'-events				50%			10%			50%
z-value		z = -9.32			z = -12.51			z = -20.85		

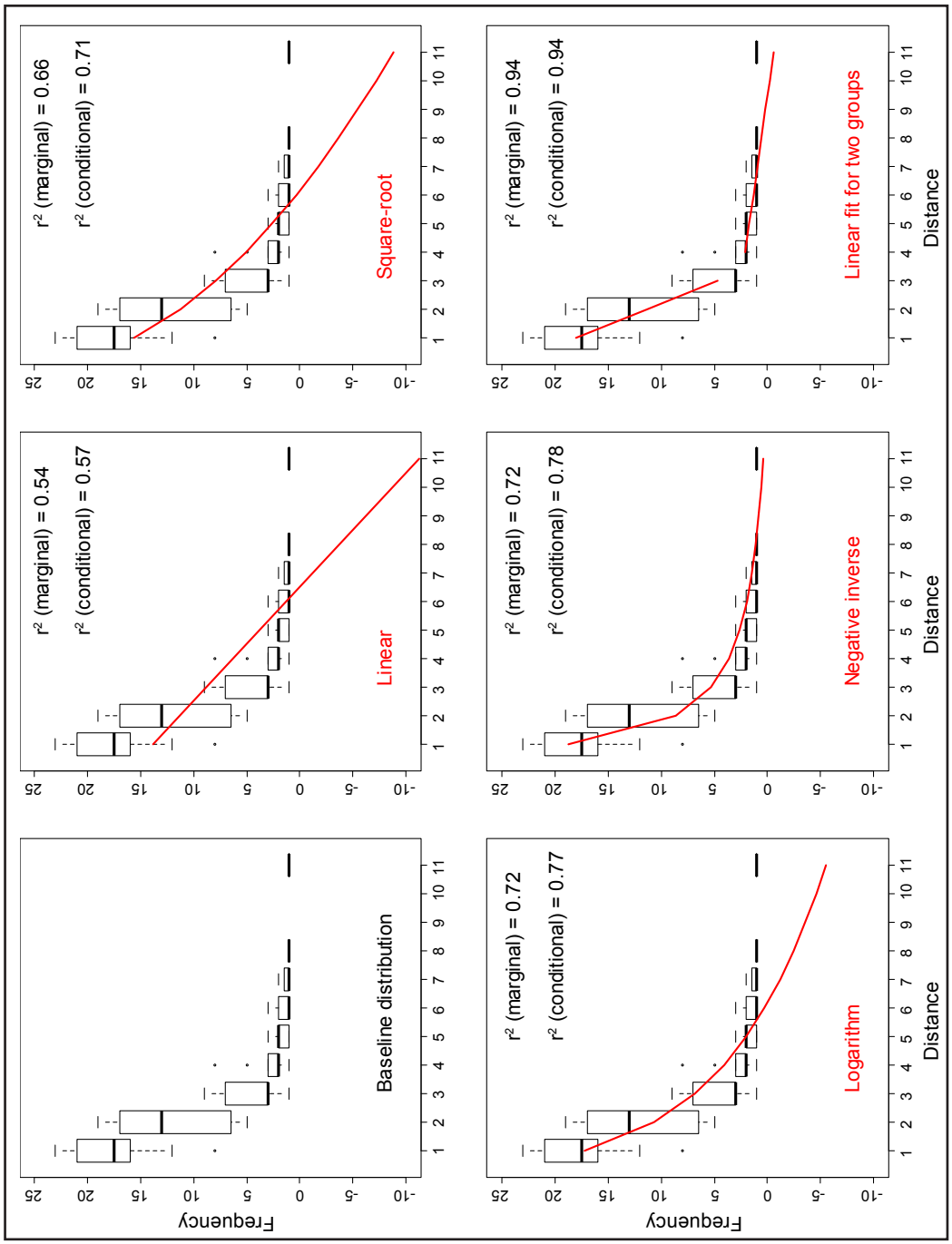


Fig. S1. Models of the relationship between breeding distance and the occurrence of EPP events. The red curve indicates the fit predicted by a linear mixed-effects model with 'Number of EPP events' as the response variable, 'year' as random factor, and 'breeding distance' (in different transformations as indicated in the figure) as the explanatory variable. R^2-values are calculated following Nakagawa & Schielzeth, 2013[1)].

[1)] Nakagawa, S., Schielzeth, H., (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models Methods in Ecology an Evolution,4: 13-1

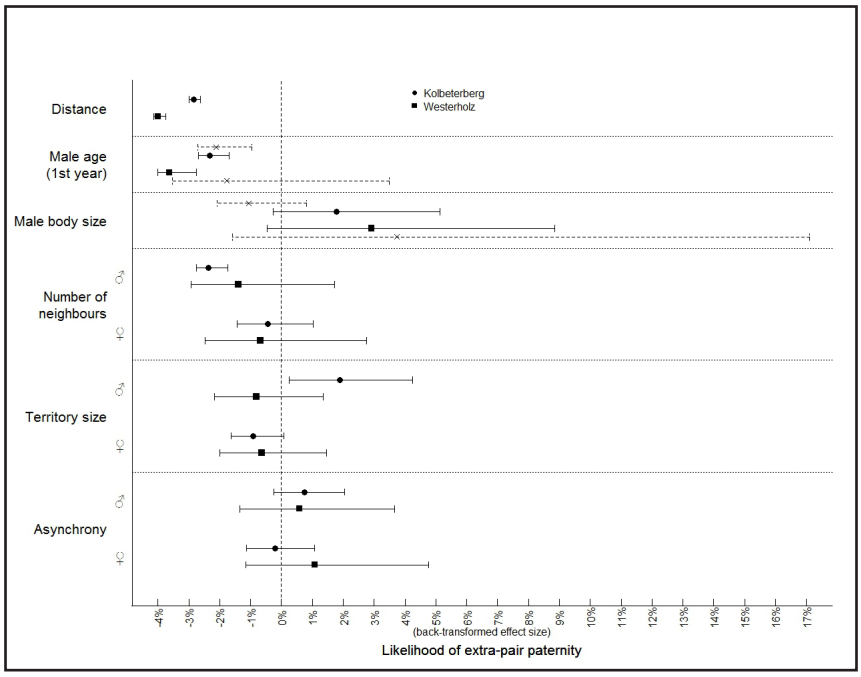


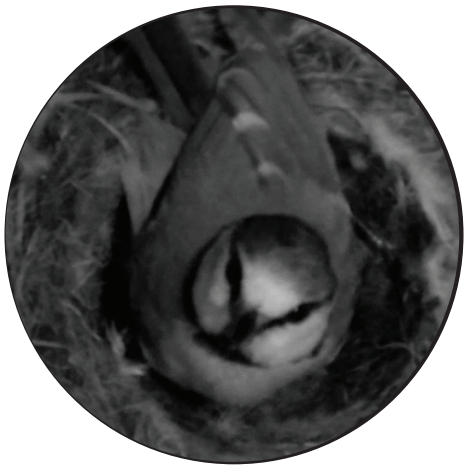
Fig S2. Effect sizes for all explanatory variables on the occurrence of extra-pair paternity in two blue tit populations. Shown are changes in EPP probabilities (back-transformed parameter estimates with subtracted intercept) and confidence intervals. Effects at higher breeding distances (interactions) are indicated by dashed error bars. See Methods and Table 2 for model details. The baseline EPP level for a yearling male and direct neighbours was 3.3% (intercept: -3.36) for Kolbeterberg and 4.1% (intercept: -3.15) for Westerholz (vertical dashed line).

Supplementary Material to Chapter 3

No relationship between female emergence time from the roosting place and extrapair paternity

Lotte Schlicht, Mihai Valcu, Peter Loës, Alexander Girg & Bart Kempenaers

Behavioral Ecology (2014), 25:650-659



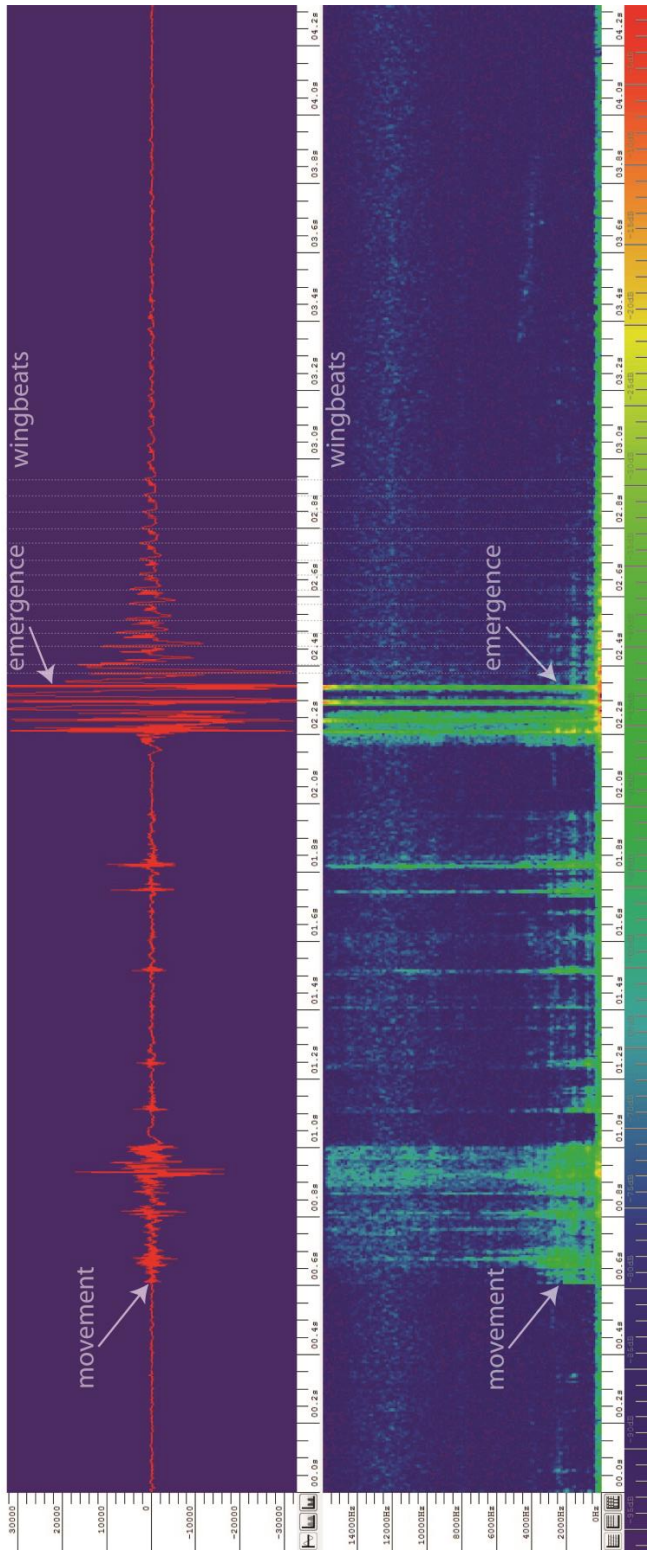


Figure S1. Waveform (top) and spectrogram (bottom) of a female emergence. In contrast to movements by the female, the emergence is a sharp noise that fills the whole spectrum. The fluttering wingbeats as the female flies away from the nestbox opening are best seen in the Waveform, but can also be detected in the spectrogram. This spectrogram was calculated using an FFT window size of 256 with an overlap of 0.5, and corresponds to the Suppl. Audio file S1.

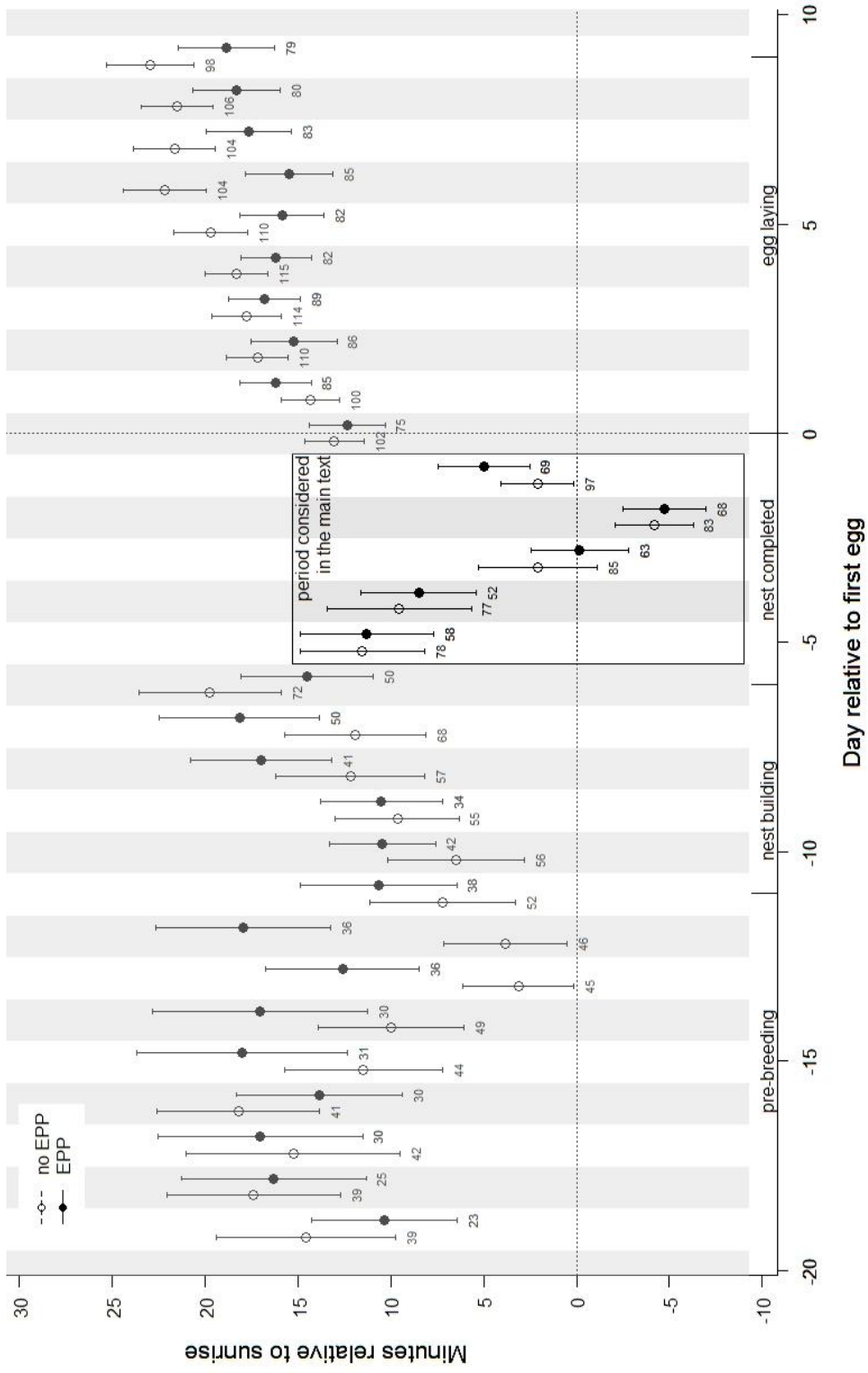


Figure S2. Difference in emergence times of females with and without extra-pair young throughout the breeding season. Shown are means and standard errors. Sample sizes are given below the bars. The indication of breeding stage above the X-axis is based on average dates for all nesting attempts between 2010 and 2013 ('pre-breeding': period before the start of nest-building; 'nest building': period between the start of nest building (at least bottom almost covered) and nest completion (nest cup with soft lining); 'egg laying': females usually lay one egg per morning with an average clutch size of ten eggs).

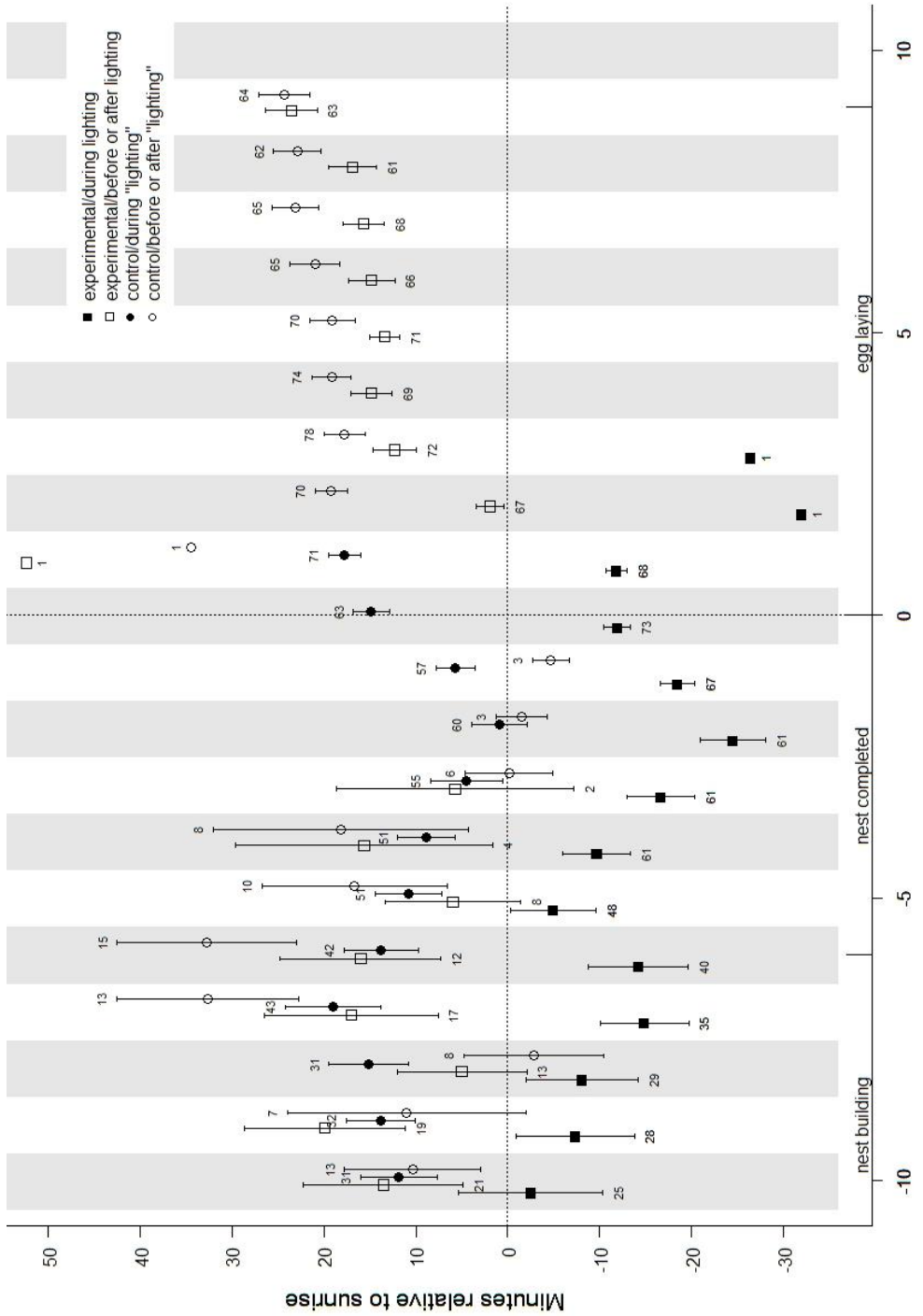


Fig. S3. Influence of the experimental light treatment on female emergence times throughout the breeding season. Shown are means and standard errors. Sample sizes are given below/above the bars. 'During lighting' data points for both control and experimental females are obtained between nest discovery and the day of the second egg, when the light treatment was applied (see Methods). A comparison of experimental and control nests during this period allows to investigate an effect of lighting on emergence time independent of year. The indication of breeding stage above the X-axis is based on average dates for all nesting attempts between 2010 and 2013 ('pre-breeding': period before the start of nest-building; 'nest building': period between the start of nest building (at least bottom almost covered) and nest completion (nest cup with soft lining); 'egg laying': females usually lay one egg per morning with an average clutch size of ten eggs).

Period	Intercept	Estimate±SE	t	P
Day -1	0.13±0.05	-0.00±0.00	-0.96	0.25
Day -2	-0.05±0.07	0.00±0.00	1.15	0.20
Days -5 to -1	-0.02±0.05	0.00±0.00	0.50	0.35

Table S1. Relationship between female emergence time and distance to the extra-pair mate. Shown are estimates from a linear mixed effects model using 'distance' (between the nestbox of the focal female and that of the female of the extra-pair mate, in meters) as the response variable, and 'emergence time' (relative to the other females on the specific day in relation to egg laying) as the explanatory variable. 'Year' and 'female identity' were included as random factors. The data were restricted to females that had extra-pair young, and to the day prior to the start of laying (day -1), two days prior to laying (day -2), and to the mean emergence on day -5 to -1, respectively.

Supplementary Material To Chapter 4

Male extra-territorial behavior predicts extra-pair paternity in blue tits, *Cyanistes caeruleus*

Lotte Schlicht, Mihai Valcu & Bart Kempenaers

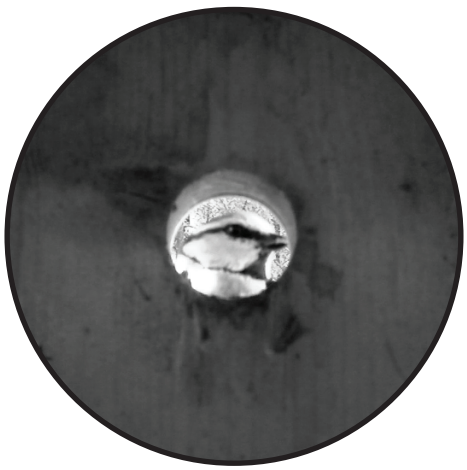


Table S1. Literature overview and sample sizes. References can be found at the end of the supplement.

Study species		Method	Sample size	Unit of observation[1)]	Reference
Scientific name	English name				
Acrocephalus arundinaceus	Great reed warbler	Radio-tracking	13	females	Bensch & Hasselquist 1992
Agelaius phoeniceus	Eastern red-winged blackbird	Observations	42	males	Westneat 1993
			57	females	
Cardinalis cardinalis	Northern cardinal	Radio-tracking	7	females	Humbird & Neudorf 2008
Chthonicola saggittata	Speckled warbler	Observations	20	males	Gardner *et al.* 2004
Cyanistes caeruleus	Blue tit	Observations	14	nests	Kempenaers *et al.* 1992
		Radio-tracking	9	individuals[2)]	Naef-Daenzer 1994
Emberiza schoeniclus	Reed bunting	Observations	24	pairs	Marthinsen *et al.* 2005
Empidonax minimus	Least flycatcher	Observations	11	females	Tarof & Ratcliffe 2000
Empidonax virescens	Acadian flycatcher	Radio-tracking	7	males	Woolfenden *et al.* 2005
			12	females	Hung *et al.* 2009
Fringilla coelebs	Chaffinch	Observations	24	pairs	Sheldon 1994, Sheldon & Burke 1994
		Radio-tracking	5	males	Hanski & Haila 1988
			9	males	Hanski 1992
			6	males	Maciejok *et al.* 1995
			3	females	
Geothlypis trichas	Common yellowthroat	Radio-tracking	13	males	Pederson *et al.* 2006
			15	females	
Hylocichla mustelina	Wood thrush	Radio-tracking	22	pairs	Evans *et al.* 2008
Icteria virens	Yellow-breasted chat	Observations	6	pairs	Mays & Ritchison 2004
		Radio-tracking	20	females	
		Automated radio-tracking	14	males	Ward *et al.* 2014
			7	females	

Study species		Method	Sample size	Unit of observation[1)]	Reference
Scientific name	English name				
Icterus galbula	Northern oriole	Observations	34	pairs	Edinger 1988
Icterus galbula bullockii	Bullock's oriole	Observations	30	pairs	Richardson & Burke 1999
Junco hyemalis	Dark-eyed junco	Radio-tracking	13	males	Chandler *et al.* 1997
			6	males	Neudorf *et al.* 2002
			13	females	
Luscinia megarhynchos	Nightingale	Radio-tracking	12	males	Naguib *et al.* 2001
Luscinia s. svecica	Bluethroat	Observations	9	pairs	Krokene *et al.* 1996
		Radio-tracking	6	females	Smiseth & Amundson 1995
Malurus cyaneus	Superb fairy-wren	Radio-tracking	7	females	Double & Cockburn 2000
Melospiza melodia	Song sparrow	Radio-tracking	12	pairs	Akçay *et al.* 2011
Oenanthe oenanthe	Wheatear	Observations	12	pairs	Currie *et al.* 1998
Parus major	Great tit	Radio-tracking	10	Individuals[2)]	Naef-Daenzer 1994
Passerina cyanea	Indigo bunting	Observations	34	pairs	Westneat 1987
			48	males	Westneat 1988
Pica pica	Black-billed magpie	Observations	25	pairs	Buitron 1983
Tachycineta bicolor	Tree swallow	Radio-tracking	4	females	Stapleton & Robertson 2006
Uria aalge	Common guillemot	Observations	22	pairs	Hatchwell 1988
Wilsonia citrina	Hooded warbler	Radio-tracking	14	females	Neudorf *et al.* 1997
			10	males	Stutchbury 1998
			19	males	Pitcher & Stutchbury 2000
			20	males	Norris & Stutchbury 2001
			23	males	Stutchbury 2005
			17	females	Chiver *et al.* 2008

[1)] Values shown in introduction are calculated by using the number of individuals (male or female) under study, respectively. If pairs or nests were observed, both members of the pair were included as individuals under study.

[2)] Sex unknown

Table S2. Percentage of blue tit pairs that sired extra-pair offspring together in relation to male age, breeding distance (neighbour order) and male or female extra-territorial nestbox visits (yes/no).

male age		1st year									2nd year and older								
breeding distance		1st			2nd			3rd			1st			2nd			3rd		
		%	N_{pairs}	N_{ind}	%	N_{pairs}	N_{ind}	%	N_{pairs}	N_{ind}	%	N_{pairs}	N_{ind}	%	N_{pairs}	N_{ind}	%	N_{pairs}	N_{ind}
male visits	yes	**0%**	9	7	**0%**	2	1	**-**	0	0	**17%**	46	35	**30%**	10	10	**0%**	1	1
	no	**2%**	203	40	**1%**	372	40	**0%**	511	40	**6%**	957	185	**2%**	1886	185	**0%**	2512	185
female visits	yes	**0%**	5	5	**0%**	1	1	**0%**	1	1	**11%**	9	7	**0%**	1	1	**0%**	1	1
	no	**2%**	365	162	**0%**	721	183	**0%**	968	185	**6%**	625	183	**2%**	1164	187	**1%**	1568	187

Based on a dataset containing all possible male-female combinations (see Methods). Shown are percentages and sample size (N_{pairs} = the number of male-female combinations, N_{ind} = the total number of involved males or females, respectively). Note that the effect of male body size is not included.

Table S3. Multiple comparisons of visitation probabilities of males and females in relation to the breeding period of the visitor or the visiting pair.

		Period defined by the first egg of the visitor			Period defined by the first egg of the visited pair		
	Focal sex	Estimate ±SE	z-value	P-value	Estimate ±SE	z-value	P-value
Late laying – Pre-laying		0.88±9.48	1.85	0.15	0.09±0.43	0.22	0.98
Late laying – Early laying	Males	1.12±0.49	2.30	0.06	0.09±0.43	0.22	0.98
Early laying – Pre-laying		0.24±0.49	0.49	0.88	0.00±0.43	0.00	1.00
Late laying – Pre-laying		0.00±0.91	0.00	1.00	1.67±0.87	1.92	0.13
Late laying – Early laying	Females	-0.41±0.90	-0.45	0.90	0.94±0.81	1.16	0.47
Early laying – Pre-laying		-0.41±0.90	-0.45	0.90	-0.73±0.87	-0.84	0.68

Multiple comparisons based on four different GLMMs with the intercept removed. Multiple comparisons are corrected for multiple testing. There was no difference between the sexes (in an interaction with sex, all P > 0.13).

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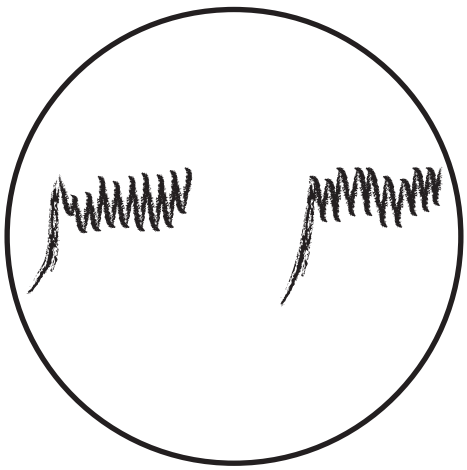
Supplementary Material to Chapter 5

Courtship calls in blue tits: occurrence throughout the day and season and their link to extra-pair paternity

Lotte Schlicht, Mihai Valcu & Bart Kempenaers



P1. List of YouTube videos of bird copulations and the presence (or absence) of potential courtship-specific vocalizations.

Species name	*Scientific name*	Courtship vocalization	Weblink
Passerines:			
Chaffinch	*Fringilla coelebs*	No	https://www.youtube.com/watch?v=1XAxZ3B8FxQ
		Yes (after 2 sec)	https://www.youtube.com/watch?v=lwRlVieD9LI
House sparrow	*Passer domesticus*	Yes	https://www.youtube.com/watch?v=MeM_hXwWaDQ
		No	https://www.youtube.com/watch?v=tDBQ_KKMtkA
Non-passerines:			
Blue-footed booby	*Sula nebouxii*	No	https://www.youtube.com/watch?v=zssfKVElWu4
Common kingfisher	*Alcedo atthis*	No	https://www.youtube.com/watch?v=t3vpWRtHAZw
Great crested grebe	*Podiceps cristatus*	Yes	https://www.youtube.com/watch?v=BnOi_1TCT1M
Common wood pidgeon	*Columba palumbus*	No	https://www.youtube.com/watch?v=mqiI8iSvttU
White Stork	*Ciconia ciconia*	No (beak rattling)	https://www.youtube.com/watch?v=mejFcMTzRPE

P2. Detailed description of experimental setup.

We designed a pilot experiment to investigate behavioural reactions of blue tits to courtship calls. We conducted a first try-out playback session at the Max Planck Institute for Ornithology, Seewiesen, 47° 58’ 21’’ N 11°14’ 8’’ O) with unstandardized playback to test the equipment, the quality of the sound recordings, and the general procedure. In the main experiment (Westerholz), we first ensured that we could identify the treated individual. Blue tits are highly territorial in early spring, and song playbacks near the nestbox are an effective method to induce approach, counter-singing and aggressive behaviour in males. We therefore played back blue tit song near a nestbox until the territorial male was in sight (and usually counter-singing). If the territorial male did not show up within 15 minutes of playback, we stopped the experiment (N = 4 cases). Only if we were certain that the territorial male was present, we switched the playback to courtship calls and observed the response of the territorial male.

Second, we ensured that the social female of the territorial male we treated was inside the nestbox. Blue tit females roost inside their nesting cavity in the majority of nights (Schlicht *et al.* 2014). One or more days before the experiment, we installed an infrared camera inside each nestbox. These cameras recorded during the night and throughout the playback session (for a description of the

camera system see Steinmeyer *et al.* (2010). We conducted the experiment before sunrise at a time when the males are engaged in the dawn chorus (e.g. Poesel *et al.* 2006), and while the females had not yet emerged (Schlicht *et al.* 2014). To this end, we arrived at the focal nestbox one hour before sunrise, 30 min before we started song playback, to avoid disturbance. Before starting the playback, we investigated whether the female was roosting inside the nestbox by inspecting the data on the memory card of the camera (inside a plastic box at the foot of the tree). If the female was not roosting inside the nestbox, we moved to the next suitable nestbox, and continued the experiment there.

The experimental treatment consisted of 15 minutes of song playback, followed by 1.5 minutes of courtship calls, when the focal male was present. We used song and courtship calls of nine different birds to avoid influences of the identity of the playback male. Courtship calls can vary in length and are often interrupted by pauses of different lengths. To standardize courtship calls among different playbacks, we produced a pattern of 15 seconds of continuous courtship calls followed by 15 seconds of silence, which we repeated three times. During the control treatment, we played back male song to attract the territorial male, followed by 1.5 minutes of silence. Control and experimental treatments were regularly distributed over the entire time span of the experiment to avoid seasonal biases. Four observers were part of the experiment. To ensure that there was no observer bias, all observers conducted at least one control and one experimental treatment, and a similar overall number of control and experimental treatments. Because the experimental period was limited due to the high breeding synchrony (see Methods), one observer could only perform one control observation. To be able to compare the different trials, we restricted the experiment to the period when the males were most territorial (nest completed, no or only few eggs laid). We conducted observations on 14 males in 11 observation sessions.

We used two mp3-players (SanDisk, Milpitas, USA and Creative, Singapore, Singapore) and three loudspeakers (frequecy range up to 20 kHz; Creative, Singapore, Singapore; ednet. ASSMANN Electronic GmbH, Lüdenscheid, Germany; JBL Professional, Northridge, USA) for the playback. The player and the loudspeakers were connected via a 10 m cable so that the playback of courtship calls could be switched on or off by the observer depending on the presence and behaviour of the territorial male. Most males did not seem disturbed by our presence. In two cases, the focal males responded to our presence by alarm calling. In these cases we moved further away until the male ceased alarming (max. 20 m distance). We did not perform experiments on one day with heavy rain.

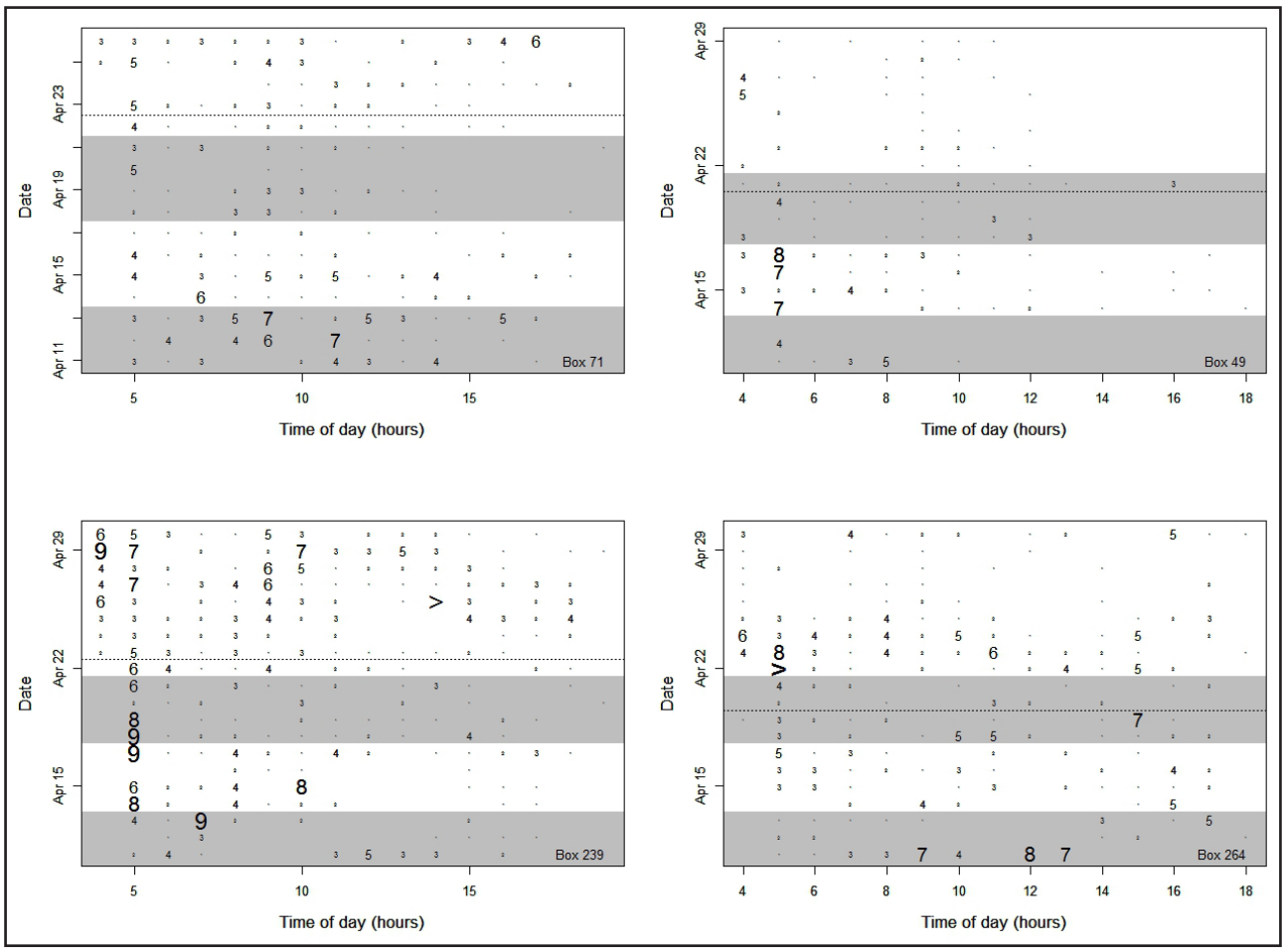


Figure S1. Distribution of courtship calls in 2013 based on preliminary analysis of 4 sound recorders. Note that files have only partially been analysed, as indicated by empty areas in the figure. The dotted line indicates the first egg in the nestbox at which the recorder was placed. The grey shading indicates rainy days. Numbers indicate the number of courtship events within the respective hour (more than 9 courtship calls are indicated by ">").

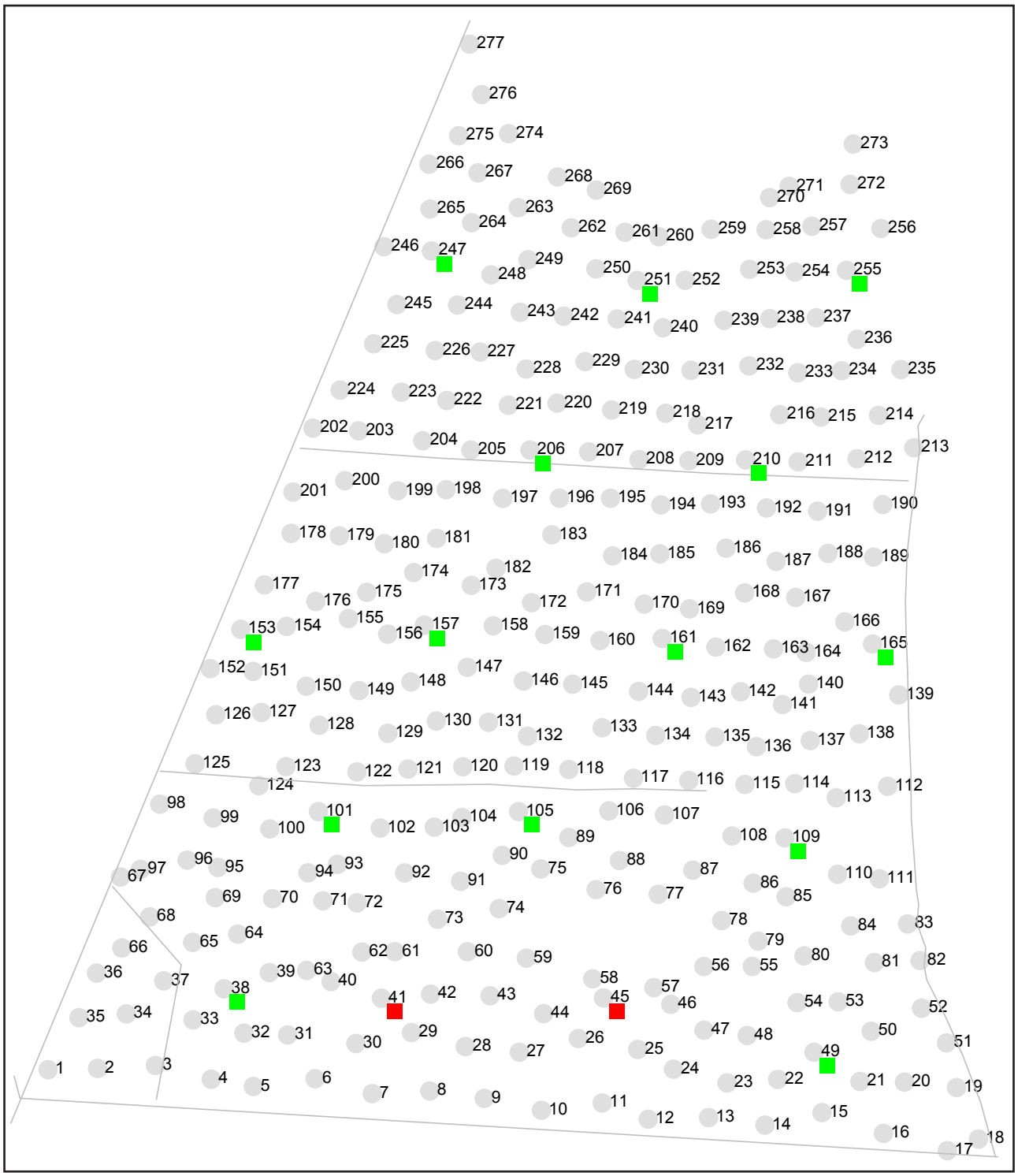


Figure S2. Locations of 16 sound recorders in 2014. The 277 nestboxes are indicated by grey circles. Green (red) squares indicate functioning (malfunctioning) sound recorders. Note that the distance between two nestboxes is on average 40 m.

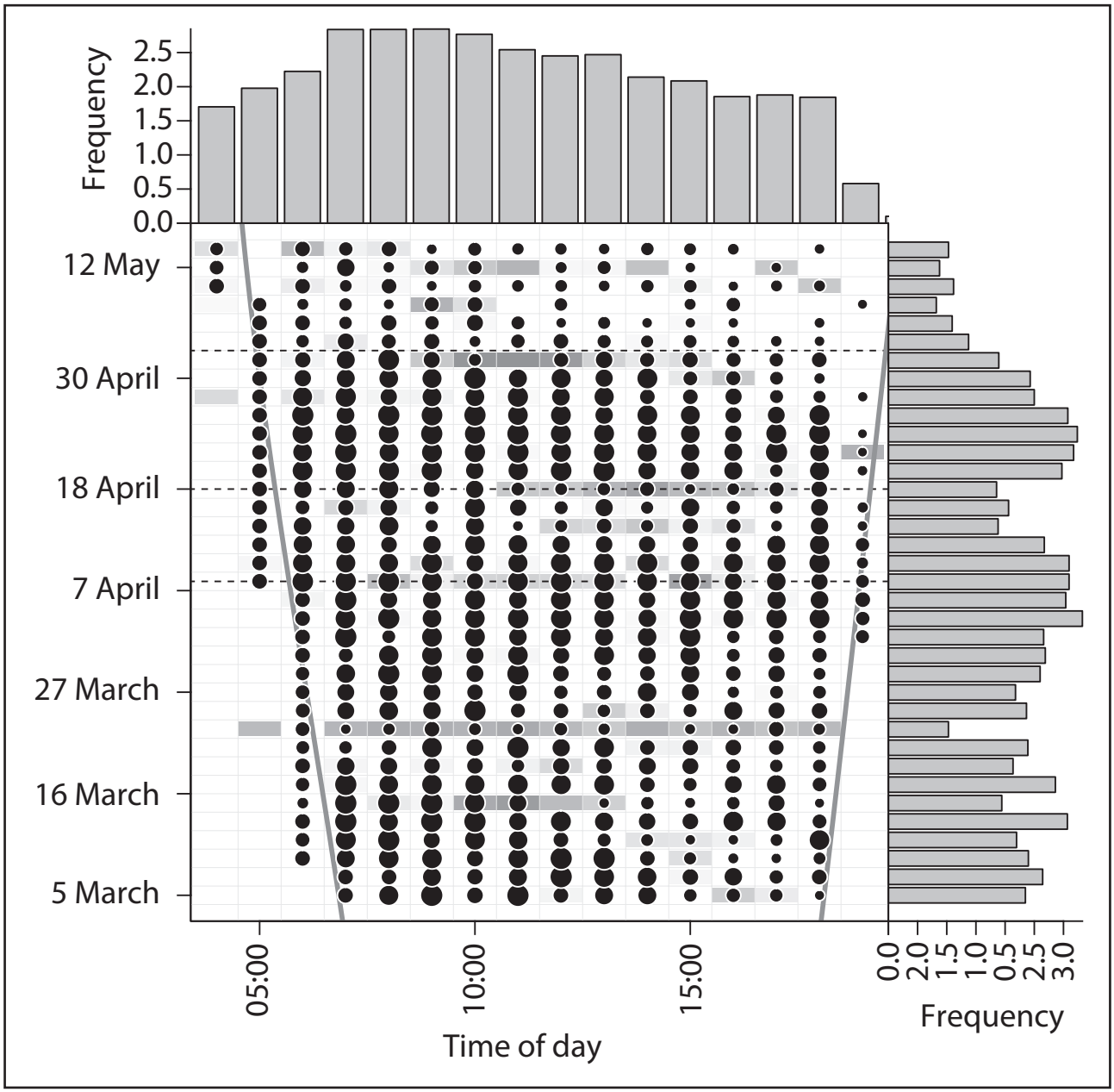


Figure S3: Daily and seasonal patterns of the occurrence of song within all files. Larger points indicate more files with song. Grey rectangles indicate rainfall, with dark rectangles indicating heavy rain throughout the forest (averaged over all files at the specific date and hour; rain scored as 'no rain', 'light rain': analysis of courtship calls unaffected, and 'heavy rain': analysis of courtship calls partly impaired). The two grey curves indicate civic sunrise and sunset, respectively. The three vertical dashed lines indicate the mean laying start, mean date of clutch completion, and mean hatching start across the population in 2014. Overall densities are shown for the time of day (right) and the season (top). We only scored for each file (length: 15 min or 30 min) whether song occurred or not. Therefore, the axes of the density plots are scaled show the average number of 15 minute-units with song per hour. Note that this graph does not show song rates. Because most files contained at least one song bout the peak of singing during the dawn chorus is not evident.

Table S1. Relationship between the number of extra-pair offspring gained and the number of potential extra-pair courtships including an interaction with "year". Model details are given in the Methods-section.

	Estimate ± SE	z-value	P-value
(Intercept; Year: 2012)	-0.19 ± 0.21	-0.92	0.36
No. courtship calls	0.13 ± 0.29	0.45	0.65
Year (2013)	-0.17 ± 0.36	-0.48	0.63
Interaction courtship calls * year (2013)	-0.29 ± 0.36	-0.80	0.42

Table S2. Relationship between the number of extra-pair offspring lost and the number of potential extra-pair courtships including an interaction with "year". Model details are given in the Methods-section. Note that including an interaction with "year" as a two/level factor strongly reduces the power of the model.

	Estimate ± SE	z-value	P-value
(Intercept; Year: 2012)	-2.90 ± 0.40	-7.20	< 0.001
No. courtship calls	-0.38 ± 0.48	-1.77	0.43
Year (2013)	-0.01 ± 0.55	0.02	0.98
Interaction courtship calls * year (2013)	-0.14 ± 0.58	-0.25	0.81

Table S3. Relationship between the number of potential extra-pair courtships and male age (first year breeder or older) including an interaction with "year". Model details are given in the Methods-section.

	Estimate ± SE	z-value	P-value
(Intercept; Year: 2012; male age: 1st year)	1.55 ± 0.10	15.00	< 0.001
Male age: Older	0.14 ± 0.16	0.88	0.38
Year (2013)	-0.22 ± 0.21	-1.08	0.28
Interaction male age (older) * year (2013)	-0.18 ± 0.31	-0.57	0.57

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CAREFUL!
!BITES!

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Lotte Schlicht
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